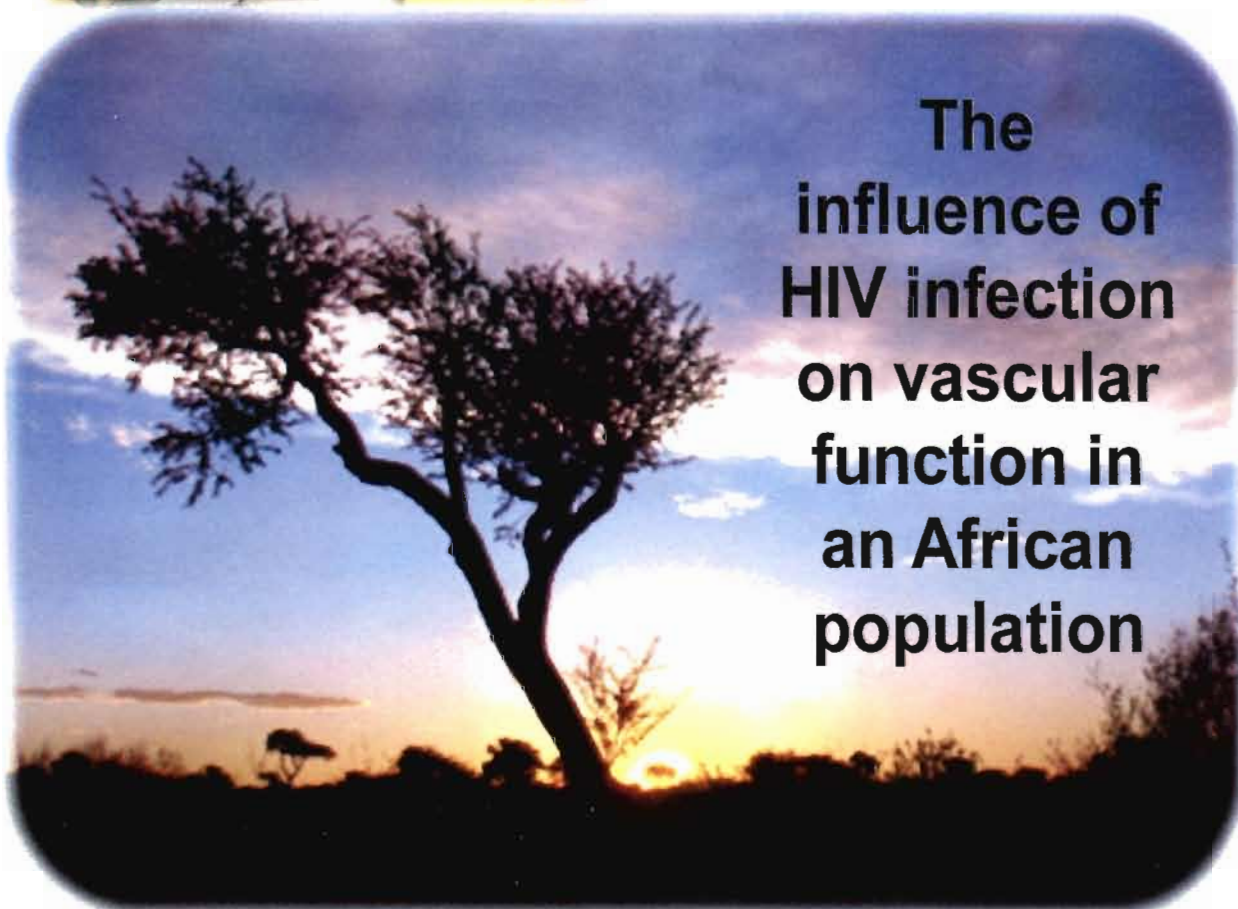




**The
influence of
HIV infection
on vascular
function in
an African
population**



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The influence of HIV infection on vascular function in an African population

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2 Corinthians 12:9

But He said to me "My grace is sufficient for you, for my power is made perfect in weakness". Therefore I will boast all the more gladly about my weaknesses, so that Christ's power may rest on me.

During the time that I worked on this study His grace to me was abundant.

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TITLE

The influence of HIV infection on vascular function in an African population

SUMMARY

Motivation

The prevention and treatment of non-communicable diseases, such as cardiovascular disease, is marginalized in South Africa by the overwhelming prevalence of human immunodeficiency virus (HIV). HIV infection and/or the treatment thereof paradoxically affects cardiovascular risk factors and may add to the burden of non-communicable diseases which are predicted to increase over the next decades.

Antiretroviral therapy was introduced in the late 1990's in industrialized countries. In South Africa the antiretroviral roll-out programme was introduced in February 2004, giving HIV infected individuals access for the first time to free antiretroviral treatment. New legislation stipulates the commencement of antiretroviral therapy at a CD4 cell count of 350 cells/mm³ instead of the current 200 cells/mm³. Although the expansion of the antiretroviral therapy will probably lead to a further decline in the HIV related mortality, the effect thereof on the burden of non-communicable diseases (e.g. cardiovascular diseases) in South Africa remains largely unknown.

There is an abundance of literature regarding research on HIV-1 infection and its cardiovascular effects in westernised Caucasian populations, but the literature concerning the HIV-1 infected black population of South Africa is lacking to a great extent. HIV-1 subtype C, prevalent in South Africa, differs genetically from subtype B, which is prevalent where most of the research was done. Furthermore, the black South African population has unique characteristics, living conditions and religious beliefs – which make extrapolation from Caucasian populations difficult. Although the clinical consequences of the subtype variations still remain unclear, no study, to our knowledge, to date has been done to evaluate the influence of HIV-1 (subtype C) infection itself, and the treatment thereof on the vascular function and cardiovascular risk of the HIV-1 infected population of South Africa.

Aim

The aim of this study (thesis) was to assess the influence of the HIV-1 subtype C infection and the antiretroviral therapy of the roll-out programme on vascular function in black Africans of the North-West Province of South Africa.

Methodology

This study is embedded in the international PURE (Prospective Urban and Rural Epidemiology) study. The PURE study is an epidemiological study that addresses questions regarding the cause and development of cardiovascular risk factors and disease within populations, particularly of low and middle income countries, including South Africa. The South African leg of the study was performed in the North West Province where a total of 2000 black South Africans (1000 urban and 1000 rural) were randomly recruited from a rural and urban setting and screened during the baseline phase in 2005. Manuscripts presented in Chapters 2 and 3, made use of the data of three hundred newly identified HIV-1 infected participants of the baseline PURE study population. These infected participants were individually matched with 300 HIV-1 uninfected participants (case-control design), according to age, gender, body mass index and locality (urban and rural). Anthropometric and cardiovascular measurements, the lipid profile, inflammatory markers and pulse wave velocity were determined. Significant differences were determined by means of independent T-tests. Analysis of covariance (ANCOVA) was performed to compare the cardiovascular variables, lipid profile, glucose, inflammatory and coagulatory markers whilst adjusting for tobacco and alcohol use. Odds ratios were calculated and partial correlations were performed whilst adjusting for mean arterial pressure (MAP), tobacco and alcohol use. The manuscript presented in Chapter 4 made use of the follow-up data obtained three years after baseline. Anthropometric measurements, the lipid profile, pulse wave velocity and carotid intima media thickness were determined. Significant differences between baseline and follow-up were determined by dependent T-tests.

All participants gave informed consent and the study was approved by the Ethics Committee of the North-West University, Potchefstroom, South Africa. The reader is referred to the methods section of each individual manuscript for a more elaborate description of the participants, study design and analytical methods.

Results and conclusions of individual manuscripts

- The aim of the first manuscript was to evaluate if HIV-1 infection itself is associated with dyslipidemia, inflammation and the occurrence of the metabolic syndrome in newly identified HIV-1 infected participants who have never received antiretroviral therapy. The results indicated that HIV-1 is associated with dyslipidemia and an inflammatory state of newly identified HIV-1 infected, never-treated, African individuals that may increase their risk for cardiovascular disease. The study showed that HIV-1 subtype C, though genetically different from subtype B, has similar association with components of the

metabolic syndrome than HIV-1 subtype B. The prevalence of the metabolic syndrome in the HIV-1 infected and uninfected participants did not differ.

- The aim of the second manuscript was to assess whether newly identified, never-treated, HIV-1 infected South Africans of African ancestry show signs of inflammatory injury of the endothelium leading to endothelial dysfunction, accelerated atherosclerosis and increased coagulation which could lead to thrombosis. The results showed that the HIV-1 infected participants had lower high-density lipoprotein cholesterol (HDL-C) levels and higher interleukin-6, C-reactive protein, intracellular adhesion molecule-1 and vascular adhesion molecule-1 levels compared to the uninfected controls. These results suggest inflammatory injury of the endothelium pointing to endothelial dysfunction. Attenuation of the protective effect of HDL-C might have worsened the endothelial inflammation. No indication of a prothrombotic state which could result in atherosclerotic disease could be detected. However, there is an indication of accelerated vascular aging and probable early atherosclerosis in the older HIV infected participants.
- The aim of the third manuscript was to determine whether soluble urokinase plasminogen activator receptor (suPAR) levels are elevated in HIV-1 infected black South Africans (treated and never-treated) compared to uninfected controls before and after a three year follow-up study. The second aim was to investigate whether suPAR levels are correlated with similar cardiovascular and metabolic changes over the three year period in the HIV-1 infected individuals, treated and never-treated. This follow-up study showed that HIV-1 infected black South Africans had significantly higher suPAR levels than uninfected controls. However, the main result of this manuscript was that treated, normo-glycemic, HIV-1 infected participants showed signs of lipodystrophy and a greater increase in suPAR levels compared to the never-treated and uninfected participants after three years. In the treated HIV-1 infected group suPAR levels were correlated positively with an increased waist circumference and negatively with TC:HDL-C ratio. No correlations were observed with either baseline CRP or IL-6. The never-treated HIV-1 infected participants showed no increase in suPAR levels in the three year time-span and a worse lipid profile compared to the uninfected controls.

Discussion

South Africa has an overwhelming prevalence of HIV and AIDS, which may, paradoxically, play a role in the predicted increase in non-communicable diseases (e.g. cardiovascular disease) over the next decades.

The influence of HIV-1 subtype C on vascular function, how it influences the risk of cardiovascular disease and how the influence is affected by the antiretroviral treatment of

the roll-out programme in HIV infected South Africans, remains largely unknown. The results of this study are, therefore, valuable in contributing to the limited knowledge regarding the probable influence of HIV-1 subtype C on vascular function. Further research should assess the contributions of HIV-1 infection itself and antiretroviral treatment to vascular dysfunction and cardiovascular disease risk.

Key words: HIV-1 subtype C, metabolic syndrome, dyslipidemia, lipodystrophy, inflammation, endothelial dysfunction, vascular aging, suPAR, antiretroviral therapy, black South Africans.

AFRIKAANSE TITEL

Die invloed van MIV infeksie op vaskulêre funksie in 'n Afrikane populasie

AFRIKAANSE OPSOMMING

Motivering

Die voorkoming en behandeling van nie-oordraagbare siektes soos kardiovaskulêre siektes, word gemarginaliseer in Suid Afrika deur die oorweldigende voorkoms van die menslike immuuniteitsvirus (MIV). MIV infeksie en/of die behandeling daarvan affekteer paradoksaal kardiovaskulêre risiko faktore en mag bydra tot die las van nie-oordraagbare siektes, wat volgens voorspellings, in die volgende paar dekades gaan toeneem:

Antiretrovirale terapie is in die laat 1990's in geïndustrialiseerde lande bekendgestel. In Suid-Afrika is die antiretrovirale voorsieningsprogram in 2004 van stapel gestuur en daardeur het die MIV geïnfekteerde individue vir die eerste keer toegang tot gratis antiretrovirale behandeling gekry. Nuwe wetgewing stipuleer die aanvang van antiretrovirale terapie vanaf 'n CD4 selteelling van 350 selle/mm³ in plaas van die huidige 200 selle/mm³. Hoewel die uitbreiding van antiretrovirale terapie waarskynlik sal lei tot 'n verdere afname in MIV verbandhoudende mortaliteit, is die effek daarvan op die las van nie-oordraagbare siektes (bv. kardiovaskulêre siektes) in Suid-Afrika nog hoofsaaklik onbekend.

Heelwat literatuur aangaande navorsing op MIV-1 infeksie en die kardiovaskulêre effek daarvan op die verwesterse Kaukasiese populasie is beskikbaar, maar daar is 'n groot tekort aan literatuur aangaande die MIV geïnfekteerde swart populasie van Suid-Afrika. MIV-1 sub tipe C, oorheersend in Suid-Afrika, verskil geneties van sub tipe B, wat voorkom waar die meeste navorsing gedoen is. Verder het die swart Suid-Afrikaanse populasie unieke karakteristieke, lewenstoestande en geloofsoortuigings – dit maak ekstrapolasie vanaf Kaukasiese populasies moeilik. Hoewel die kliniese uitwerking van die sub tipe variasies nog onseker is, is daar volgens ons kennis tot op datum geen studie gedoen om die invloed van die MIV-1 (sub tipe C) infeksie self, of die behandeling daarvan, op vaskulêre funksie en kardiovaskulêre risiko van die MIV-1 geïnfekteerde populasie van Suid-Afrika vas te stel nie.

Doelstelling

Die doel van hierdie studie (tesis) is om die invloed van die HIV-1 sub tipe C infeksie en die antiretrovirale terapie van die voorsieningsprogram op die vaskulêre funksie van die swart Afrikane van die Noordwes Provinsie van Suid-Afrika vas te stel.

Metodologie

Hierdie studie vorm deel van die groter internasionale PURE (Prospective Urban and Rural Epidemiology) studie. Die PURE studie is 'n epidemiologiese studie wat vrae aanspreek aangaande die oorsaak en ontwikkeling van kardiovaskulêre risiko faktore en siekte binne populasies, veral van laer en middel inkomste lande, wat Suid-Afrika insluit. Die Suid-Afrikaanse deel van die studie is uitgevoer in die Noordwes Provinsie waar 'n totaal van 2000 swart Suid-Afrikaners (1000 stedelik en 1000 landelik) ewekansig gewarf is van 'n stedelike en landelike gebied en ondersoek is tydens die basislyn fase in 2005. In die manuskripte wat in Hoofstuk 2 en 3 vervat is, word gebruik gemaak van die data van driehonderd nuut geïdentifiseerde MIV-1 geïnfekteerde deelnemers van die basislyn PURE studie populasie. Hierdie geïnfekteerde deelnemers is individueel gepas met 300 nie-geïnfekteerde MIV deelnemers (geval-kontrole ontwerp) volgens ouderdom, geslag, liggaamsmassa-indeks en lokaliteit (stedelik en platteland). Antropometriese- en kardiovaskulêre metings, die lipied profiel, inflammatoriese merkers, en polsgolf snelheid is bepaal. Betekenisvolle verskille is bepaal deur onafhanklike T-toetse. Die kovariansie analise (ANKOVA) is uitgevoer om die kardiovaskulêre veranderlikes, lipied profiel, glukose vlakke, inflammatoriese en koagulatoriese merkers te vergelyk, terwyl gekorrigeer is vir tabak en alkohol gebruik. Waarskynlikheidsverhoudings is bereken en partiële korrelasies uitgevoer, terwyl gekorrigeer is vir gemiddelde arteriële druk, tabak en alkohol gebruik. Die manuskrip wat in Hoofstuk 4 vervat is, maak gebruik van die opvolgdata wat 3 jaar na die basislyn opname versamel is. Antropometriese en kardiovaskulêre metings, die lipied profiel, polsgolf snelheid en karotis intima media dikte is bepaal. Betekenisvolle verskille tussen die basislyn en opvolg opnames is bepaal met afhanklike T-toetse.

Alle proefpersone het ingeligte toestemming verleen en die studie is goedgekeur deur die etiek komitee van die Noordwes Universiteit, Potchefstroom, Suid-Afrika. Die leser word verwys na die metode afdeling van elke individuele manuskrip vir 'n meer breedvoerige beskrywing van die deelnemers, studie ontwerp en analitiese metodes.

Resultate en gevolgtrekkings van die individuele manuskripte

- Die doel van die eerste manuskrip is om vas te stel of MIV-1 infeksie self geassosieer word met dislipidemie, inflammasie en die voorkoms van die metaboolse sindroom in nuut-geïdentifiseerde MIV-1 geïnfekteerde deelnemers wat nog nooit antiretrovirale terapie ontvang het nie. Die resultate het getoon dat MIV-1 geassosieer word met dislipidemie en inflammasie van nuut-geïdentifiseerde MIV-1 geïnfekteerde, nooit-behandelde Afrikaners wat hul risiko vir kardiovaskulêre siektes mag verhoog. Die studie toon dat MIV-1 sub tipe C, hoewel geneties verskillend van sub tipe B, dieselfde

assosiasies met komponente van die metaboliese sindroom toon as MIV-1 sub tipe B. Die algemene voorkoms van die metaboliese sindroom in geïnfekteerde en nie-geïnfekteerde deelnemers het nie verskil nie.

- Die doel van die tweede manuskrip was om vas te stel of nuut-geïdentifiseerde, nooit-behandelde MIV-1 geïnfekteerde Suid-Afrikaners van Afrika oorsprong tekens toon van inflammatoriese besering van die endoteel wat lei tot endoteel disfunksie, versnelde aterosklerose en verhoogde koagulasie wat tot trombose kan lei. Die resultate toon dat die MIV-1 geïnfekteerde deelnemers laer vlakke hoë-digtheid lipoproteïen cholesterol (HDL-C) en hoër vlakke interleukin-6, C-reaktiewe proteïen, intrasellulêre adhesiemolekuul-1 en vaskulêre adhesiemolekuul-1 het in vergelyking met die nie-geïnfekteerde deelnemers. Hierdie resultate suggereer inflammatoriese besering van die endoteel wat dui op endoteel disfunksie. Attenuasie van die beskermende effek van HDL-C mag die inflammasie van die endoteel vererger. Geen indikasie van 'n protrombotiese toestand wat kan lei tot aterosklerotiese siekte kon aangetoon word nie. Daar was egter 'n indikasie van versnelde vaskulêre veroudering en waarskynlik vroeë aterosklerose in die ouer geïnfekteerde deelnemers.
- Die doel van die derde manuskrip is om vas te stel of oplosbare urokinase plasminogeen aktivator reseptor (suPAR) vlakke verhoog is in MIV-1 geïnfekteerde swart Suid-Afrikane (behandel en nooit-behandel) in vergelyking met nie-geïnfekteerde kontrole deelnemers voor en na 'n drie jaar opvolg studie. Die tweede doel is om te ondersoek of die suPAR vlakke korreleer met dieselfde kardiovaskulêre en metaboliese veranderinge oor drie jaar in die MIV-1 geïnfekteerde individue, behandel en nooit-behandel. Hierdie opvolg studie toon dat MIV-1 geïnfekteerde swart Suid-Afrikane betekenisvolle hoër suPAR vlakke het as die nie-geïnfekteerde kontrole deelnemers. Die hoofresultaat van die manuskrip is egter dat behandelde normo-glisemiese, MIV-1 geïnfekteerde deelnemers tekens van lipodistrofie en 'n groter toename in suPAR vlakke toon as die nooit-behandelde en nie-geïnfekteerde deelnemers na drie jaar. In die behandelde MIV-1 geïnfekteerde groep het die suPAR vlakke positief gekorreleer met 'n toename in middelomtrek en negatief met die totale cholesterol:HDL-C ratio. Geen korrelasies is egter waargeneem met basislyn IL-6 of CRP nie. Die nooit-behandelde MIV-1 geïnfekteerde deelnemers het geen toename in suPAR vlakke oor die tydperk van drie jaar getoon nie en hul lipied profiel is swakker in vergelyking met die nie-geïnfekteerde deelnemers.

Bespreking

Suid-Afrika het 'n oorweldigende voorkoms van MIV en Vigs wat, paradoksaal, 'n rol in die voorspelde verhoogde voorkoms van nie-oordraagbare siektes (bv. kardiovaskulêre siektes) mag speel.

Die invloed van MIV-1 sub tipe C op vaskulêre funksie, hoe dit die risiko vir kardiovaskulêre siektes beïnvloed en hoe hierdie invloed geaffekteer word deur die antiretrovirale behandeling van die voorsieningsprogram in MIV geïnfekteerde Suid-Afrikane bly grootliks onbekend. Die resultate van hierdie studie lewer daarom 'n waardevolle bydrae tot die beperkte kennis aangaande die waarskynlike invloed van MIV-1 sub tipe C op vaskulêre funksie. Verdere navorsing behoort die bydrae van MIV-1 infeksie self en die antiretrovirale behandeling tot vaskulêre disfunksie en kardiovaskulêre risiko vas te stel.

Sleutel woorde: MIV-1 sub tipe C, metaboliese sindroom, dislipidemie, lipodistrofie, endoteel disfunksie, vaskulêre veroudering, oplosbare urokinase plasminogeen aktivator reseptor, antiretrovirale terapie, swart Suid-Afrikane.

PREFACE

For the purpose of this study it was decided to use the article format. Therefore, Chapters 2, 3 and 4 are manuscripts in the form of articles. All manuscripts were submitted for publication in peer reviewed journals with Chapter 2 already being published. Although the appropriate and relevant literature backgrounds are discussed in each separate manuscript, Chapter 1 also gives an additional, more elaborate literature survey. In all of the manuscripts the promoter and co-promoter are named as co-authors, as well as persons who participated in the initial concept and design of the PURE study. However, the main and first author initiated and was responsible for most stages of each manuscript, including literature searches, the organisation and coordination of the follow-up study, collection of data, statistical analysis, interpretation of results and the writing of the manuscripts. The co-authors acted in their roles as promoter and co-promoter. All co-authors gave consent that the manuscripts could be used in this thesis.

The first manuscript was submitted to and published in *Lipids*, the second to *The Cardiovascular Journal of Africa* and the third manuscript was submitted to *Journal of Infection*. The relevant references are provided at the end of each chapter according to the author's instructions of the specific journal to which the manuscripts were submitted for publication.

AUTHOR'S CONTRIBUTIONS

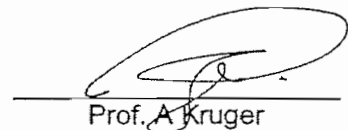
Name	Role in the study
Mrs. CMT Fourie M.Sc.	Responsible for literature searches, conception and design of the study, the organisation and coordination of the follow-up study, collecting of cardiovascular data, processing of data, analysis of IL-6, statistical analysis, design and planning of manuscripts, interpretation of results and writing of all manuscripts.
Prof. JM van Rooyen D.Sc.	Promoter. Conception and design of the study, supervised the writing of manuscripts, acquisition of cardiovascular data, revised thesis critically for important intellectual content.
Prof. AE Schutte Ph.D.	Co-promoter. Conception and design of the study, supervised the writing of manuscripts, acquisition of cardiovascular data, revised thesis critically for important intellectual content.
Prof. A Kruger Ph.D.	Principal investigator of PURE South Africa study, data acquisition, contributed in the statistical analysis (third article) and writing of the methods.
Prof. M Pieters Ph.D.	Gave guidance in interpretation of coagulation data and gave valid scientific input in second manuscript.
Dr. K Conradie Ph.D.	Analysis of IL-6 data, gave guidance in interpretation of IL-6 data in second manuscript.
Dr. T Hoekstra Ph.D	Analysis of coagulation data, gave guidance in interpretation of coagulation data and gave valid scientific input in second manuscript.
Dr. M H Olsen Ph.D.	Gave guidance in interpretation of suPAR data and revised the third manuscript critically for important intellectual content.
Dr. J Eugen-Olsen Ph.D.	Gave guidance in interpretation of suPAR data and revised the third manuscript critically for important intellectual content.

The following is a statement from the co-authors confirming their individual role in each study and giving their permission that the three manuscripts may form part of this thesis.

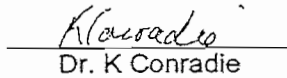
I declare that I have approved the above-mentioned manuscripts, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give my consent that they may be published as part of the Ph.D. thesis of Mrs. Carla Fourie.

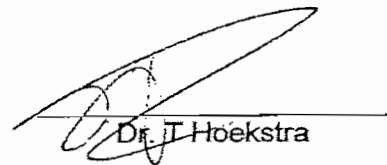

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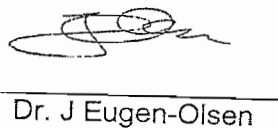

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ABBREVIATIONS

AIDS	acquired immune deficiency syndrome
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ART	antiretroviral therapy
ARV	antiretroviral
ATP III	Adult Treatment Panel III
BMI	body mass index
CAM	cell adhesion molecules
CI	confidence intervals
CRP	C-reactive protein
CSWA	cross sectional wall area
CV	cardiovascular
CVD	cardiovascular disease
DBP	diastolic blood pressure
gp	glycoprotein
GPI	glycosyl-phosphatidylinositol
HAART	Highly Active Antiretroviral Therapy
HDL-C	high-density lipoprotein cholesterol
HIV	human immunodeficiency virus
ICAM	intracellular adhesion molecule
IDF	International Diabetes Federation
IL-1	interleuken-1
IL-6	interleuken-6
IMT	intima-media thickness
LDL	low-density lipoprotein
MAP	mean arterial pressure
MetS	metabolic syndrome
N	number of participants
NNRTI's	non-nucleoside reverse transcriptase inhibitors
NO	nitric oxide
NRTI's	nucleoside reverse transcriptase inhibitors
OR	odds ratios
PAI-1	plasminogen activator inhibitor-1
PI's	protease inhibitors
PP	pulse pressure

PURE study	Prospective Urban and Rural Epidemiology study
PWV	pulse wave velocity
SBP	systolic blood pressure
suPAR	soluble urokinase plasminogen activator receptor
Tat	transactivator of transcription
TC	total cholesterol
TG	triglycerides
TNF- α	tumor necrosis factor alpha
uPAR	urokinase plasminogen activator receptor
VCAM	vascular adhesion molecule
vWF	von Willebrand factor
WC	waist circumference
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

GENERAL INTRODUCTION

Since the introduction of Highly Active Antiretroviral Therapy (HAART), human immunodeficiency virus (HIV) infection, although fatal, has become a chronic and manageable disease¹ and the life expectancy for HIV infected individuals has increased. Besides some very uncomfortable side effects due to the infection and the therapy, another more serious side effect has emerged, namely an increased risk for cardiovascular disease (CVD).^{2,3} The prevalence of hypertension is not increased in the HIV infected population,⁴ but cardiovascular involvement in various forms, such as dyslipidemia,^{5,6} lipodystrophy,^{7,8} endothelial dysfunction,^{2,9} accelerated atherosclerosis¹⁰ and coagulation disorders¹¹ have been documented worldwide among HIV infected people.

In HIV infected individuals' CVD and vascular dysfunction can be associated with various factors, such as the infection itself, opportunistic infections, the therapy or non HIV related cardiovascular risk factors (such as smoking or age).¹² It is well known that the lipid profile of HIV infected people changes, and these changes include increased levels of triglycerides (TG), and decreased levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C).^{3-6,13-15} Since the introduction of HAART, an increase in LDL-C is seen in HIV infected persons^{3,14,16} and the therapy has been associated with insulin resistance, glucose intolerance, unfavourable fat distribution and dyslipidemia.^{16,17} The above metabolic abnormalities are among the most significant side effects experienced with the antiretroviral therapy, in particular with protease inhibitors.^{8,12,18} The changes seen are reminiscent of the metabolic syndrome,⁸ which has been identified as a significant and multifaceted risk factor for CVD.^{16,19} However, it remains unclear what the relative contribution of the infection (and ongoing inflammation), the antiretroviral therapy, and the interaction between them is to the above mentioned dyslipidemic changes.^{2,20}

Lipodystrophy, a term describing varying degrees of fat redistribution, is increased in HIV infected people and is associated with an increased risk of developing CVD.^{15,16} HIV lipodystrophy is an insulin resistant, dyslipidemic state.²¹ The etiology of these lipodystrophic changes are uncertain, and although it is mostly reported in individuals with treated HIV infection,^{7,16} it may also be the result of the inflammation as response to the virus or an effect of the virus itself.¹⁶

Endothelial dysfunction is seen in HIV infected people, and this is a key event in the development of CVD.⁹ Endothelial dysfunction can be characterized by a change in the normal endothelial functions from vasorelaxant, anticoagulant, antiplatelet and profibrinolytic, to vasoconstrictive, procoagulant, platelet-activating and antifibrinolytic functions.⁹ An

increase in cardiovascular risk is seen in the HIV infected population and endothelial dysfunction, impaired fibrinolysis and excess inflammation seem to contribute to this increased risk.¹⁶ It seems that HIV infection itself produces vascular inflammation, which appears to be an important step in the development of a fatty streak, the precursor of atherosclerotic plaque in the development of atherosclerosis.³ Atherosclerosis is believed to be an inflammatory disease²² and evidence indicates that inflammation derived from various risk factors such as bacterial and viral infections (e.g. HIV), abdominal obesity, dyslipidemia, hyperglycemia and hyperhomocysteinemia play major roles in the development of endothelial dysfunction and atherosclerosis.²³ These risk factors are all associated with inflammation.²³ HIV infection is also associated with the activation of inflammatory pathways in the vascular wall^{3,24} and this may increase the risk and complications of atherosclerosis and related cardiovascular events.

Atherosclerosis is a clinically silent disease,² it is a slow process, and its incidence increases with age.²⁵ Biological markers such as the cell adhesion molecules (CAM) give an indication regarding the development of atherosclerosis and the development of vascular dysfunction. An increased concentration of cell adhesion molecules, as a result of endothelial dysfunction, has been reported in HIV-positive patients^{3,23} where both intracellular adhesion molecule (ICAM) and vascular adhesion molecule (VCAM) levels were elevated.⁹ Atherosclerotic cardiovascular events are commonly manifested via a thrombotic event leading to adverse effects,²⁶ and a wide range of coagulation disorders may be associated with HIV infection itself.²⁷

Several cardiovascular risk factors have been associated with, or seen in the HIV infected population since the longer life expectancy due to HAART.^{3,24} However, there is still uncertainty about the relative contribution of the infection, the virus itself, the associated inflammatory response, the antiretroviral therapy, and the interaction between them to these cardiovascular risk factors.² Still, Hsue *et al.*, reason that HIV infection itself should count as a coronary risk factor, similar to the traditional cardiovascular risk factors (such as smoking, hypertension, hypercholesterolemia, and diabetes).¹⁰

The majority of studies done on HIV-1 have been done on subtype B. The prevalent subtype in sub-Saharan Africa is subtype C which accounts for 55-60% of all HIV infections worldwide.²⁸ Subtype C differs as much as 30% in its genome from subtype B²⁸ and whether the effect thereof on the vascular system would be the same as subtype B is uncertain.

LITERATURE OVERVIEW

1. Human immunodeficiency virus (HIV) infection

1.1. The HIV epidemic in sub-Saharan Africa

It is estimated that there are 24.5 million adults and children living with HIV-1 in sub-Saharan Africa²⁹ and the epicentre of this pandemic is Southern Africa^{29,30} where an estimated 5.5 – 6.1 million infected people are living.³¹ Antiretroviral therapy (ART) has transformed HIV-1 infection from a still fatal condition to a chronic, manageable disease.^{1,11} Although prevention programmes have slowed the spread of the virus in South Africa³² and the ART roll-out programme was introduced in February 2004,³³ these therapies are still not available to all the HIV infected people of South Africa.³⁴

Phylogenetic analysis revealed that HIV-1 strains can be subdivided into groups (M,N and O), subtypes, sub-subtypes and circulating recombinant forms.^{35,36} All of the HIV-1 strains worldwide responsible for the pandemic belong to the M (Major) group,³⁶ and there are nine subtypes of HIV-1 group M namely A-D, F-H J and K.^{35,36} Globally the predominant forms are subtypes A and C, followed by subtype B.³⁶ Although subtype B represents only a limited proportion of infections worldwide,³⁶ it is by far the most common in North America, Europe and Australia, and was mostly represented among the first strains characterized.^{28,36} The literature reveals that up to date the most studies on HIV-1 have been done on subtype B. The vast majority of infections, however, take place in Africa where the prevalent subtypes are mainly C (which account for 55-60% of all HIV infections worldwide), A and G,^{28,30} and these subtypes differ as much as 30% in their genomes from subtype B.²⁸ These multiple subtypes show significant differences in functions and increasing evidence suggests that different HIV-1 subtypes might exhibit disparate biological behaviours. At an earlier stage of the HIV epidemic in South Africa, subtype B viruses were identified among homosexual men who reported contacts with the United States,³⁷ but in the expanding South African epidemic the predominant virus seen in infected people is HIV-1, group M, subtype C.^{31,32,36-38}

1.2. The human immunodeficiency virus (HIV)

The HI virus, an enveloped RNA virus and a member of the lentivirus family has two strains, HIV-1 and HIV-2.³⁹ HIV-1 is responsible for most of the international epidemic (as seen in the previous paragraph) while HIV-2, which remains uncommon in North America, arose in West Africa and passed with immigration into Europe.³⁹ In persons infected with HIV-2, immunodeficiency seems to develop more slowly and to be milder.³⁹

A gradual destruction of the CD4 T lymphocyte populations is characteristic of HIV-1 infection with acquired immune deficiency syndrome (AIDS) being the last stage of the disease.³⁰ The viremia peaks during the first weeks (the primary or seroconversion phase), but HIV-1 replication is dynamic throughout the disease.^{30,40} Disease progression will depend on the viral load and CD4 cell count in the blood.⁴⁰ A strong cytotoxic T cell response will lower the levels of the circulating virus³⁹ and progression to the final AIDS phase takes years.^{30,40} Depending on the CD4 counts an infected person progresses through an asymptomatic phase (CD4 between 500-800 cells/mm³), a symptomatic phase where opportunistic infections occur and the last phase known as AIDS where the CD4 count is below 200 cells/mm³.^{30, 39,40}

The glycoproteins (gp) 120 on the surface of the HI virus have an affinity for the cluster designation 4 proteins (CD4, a 58 kDa monomeric glycoprotein)⁴¹ produced and displayed on the surface of selected cells of the body, with high surface expression on helper T cells (which are referred to as CD4 cells), and to a lesser extent on monocyte/macrophage cells (in the loose connective tissue of the gastrointestinal and genital mucosa), dendritic cells (residing within lymph nodes and nodules) and some B cells.^{39,41} Binding of the gp 120 on the CD4 molecule receptor needs binding to a co-receptor (chemokine receptor) to increase the contact between the HIV-1 particle and the surface of the cell with which it is about to merge.³⁹ After merging, virus replication takes place causing an intense attack on HIV infected CD4 cells by specific cytotoxic T cells and paralyzing the immune system when CD4 cells are depleted.³⁹ The number of CD4 cells, therefore, reveals the degree of immunodeficiency, while the viral load determines the rate of destruction of the immune system.³⁰ Viral replication is promoted in infected cells, and augmented in cells already producing the virus by an abundant production of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α).⁴² Activation of the inflammatory pathways may be specifically an effect of the transactivator of transcription (Tat) protein,³ a nuclear regulatory protein which plays a crucial role in viral replication and is actively released from HIV infected cells.⁴³ During acute infection of T cells by HIV-1, Tat is released from the cells in an active form,⁴⁴ is probably an important mediator of the inflammatory responses^{3,45} and induces the cell surface expression of ICAM-1 and VCAM-1 in endothelial cells. This suggests that it may induce the interaction of endothelial cells with leukocytes.⁴⁶ It is possible that this Tat-induced inflammatory environment in the vascular endothelium may contribute to accelerated development of atherosclerosis.²⁴

1.3. Highly Active Antiretroviral Therapy for HIV

The use of antiretroviral drugs slows down the replication of the virus and can greatly enhance the quality of life, but it does not eliminate the virus.⁴² Although low CD4 counts are associated with a variety of conditions, including viral, bacterial and parasite infections, sepsis, tuberculosis, burns, trauma, malnutrition, over-exercising, pregnancy, corticosteroid use, normal daily variation, psychological stress, and social isolation,⁴⁷ in HIV infected people it reveals the degree of immunodeficiency and is used to assess the stage of infection.³⁰ Low CD4 counts (≤ 200 cells/mm³)¹⁰ together with clinical manifestations (e.g. occurrence of opportunistic infections) are the key criteria to assess the stage of infection³⁰ and to begin antiretroviral therapy. Early and aggressive antiretroviral therapy helps people maintain a more effective cytotoxic T cell response, leads to an immediate decrease in viral load^{39,42} and over all better quality of life. The plasma viral load is widely used to monitor the therapeutic success of the antiretroviral therapy.³⁰

There are three main classes of antiretroviral drugs namely nucleoside reverse transcriptase inhibitors (NRTI's), the first drugs which were to be given as antiretroviral agents, and which interfere with the HIV replication by becoming incorporated into viral DNA in the place of the normal DNA building block.⁸ Non-nucleoside reverse transcriptase inhibitors (NNRTI's) are structurally diverse, cause a conformational change in the enzyme,⁸ thus directly inhibit the functioning of the reverse transcriptase enzyme essential for the early stages of HIV replication. Protease inhibitors (PI's) act by inhibiting the action of the HIV protease enzyme,^{8,39,40} and though it does not prevent production of new viral copies, it prevent the formation of new viruses,^{39,40} as those copies produced are unable to infect new cells.⁸ The development of HIV-specific PI's has given hope to people infected with HIV-1.³⁹ Therapy with multi drug combinations (PI's, combined with NRTI's and NNRTI's) known as HAART,⁸ greatly improves the suppression of the HIV particle production, increases the CD4 counts and has an impact on preserving an intact cytotoxic T-cell response³⁹ provided the patient adheres to the treatment regimens.⁸

Although antiretroviral therapy, especially HAART, has many benefits like improving life expectancy and quality of life of people infected with HIV, this is not without cost.^{8,48} As new therapies shifted some of the clinical focus from acute care to more chronic issues, new clinical issues are the threatening side effects of the therapeutic efforts.¹⁵ Metabolic abnormalities, such as hyperlipidemia and central obesity, increases in triglyceride and cholesterol levels and insulin resistance (hyperglycaemia), are among the most significant side effects experienced with antiretroviral therapy, in particular with PI's.^{8,12,18} The antiretroviral therapy alters the lipid profiles⁴⁹ and enhances the risk of cardiovascular events

as a result.¹⁸ The adverse effects associated with antiretroviral medication use, but also the long term effects of the infection itself (as HAART cannot completely eradicate HIV-1), have become increasingly problematic.⁵⁰ This is seen in the study of Bonfanti *et al.*, where the prevalence of metabolic syndrome was greater in the HIV-infected population compared to the general population, but was similar in treated and untreated HIV-positive subjects.⁵¹ In HIV infected people the development of premature atherosclerosis is an emerging threat.² HAART directly promotes atherosclerosis independently from the metabolic changes and people treated with PI's show higher prevalence of atherosclerotic lesions in the carotid arteries than HIV infected people naïve from PI treatment.¹² According to van Wijk *et al.*, both antiretroviral therapy and HIV infection may promote atherosclerosis through mechanisms involving endothelial cells, either directly or indirectly via metabolic risk factors.⁵²

In South Africa, the antiretroviral roll-out programme was introduced in February 2004 giving HIV infected individuals access for the first time to free antiretroviral treatment.³³ The antiretroviral therapy is started at a CD4 cell count of ≤ 200 cells/mm³, and as many developing countries, South Africa uses the World Health Organization's recommendation of two nucleoside reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI) as first-line therapy.^{33,53}

2. Cardiovascular disease

2.1. Cardiovascular disease in South Africa

The prevalence of communicable diseases like HIV and tuberculosis is very high in South Africa and this place a heavy burden on the health system. This burden is paralleled by a growing threat of non-communicable diseases⁵⁴ such as cardiovascular disease, type 2 diabetes, cancer, chronic lung disease and depression.⁵⁵ Coronary artery disease, hypertensive heart disease and stroke already account for more than a third of deaths in people older than 65 years in South Africa.⁵⁶ Hypertension is a widespread problem in South Africa and has a high prevalence in urban (56%),⁵⁶ as well as in more rural (20-23%) areas.⁵⁷

Black Africans are more likely to be diagnosed with heart failure and less likely to be diagnosed with coronary artery disease.⁵⁶ In fact, atherosclerotic disease is historically nearly absent in black Africans.⁵⁸ However, atherosclerosis is increasingly seen in patients with cardiovascular disease in South Africa⁵⁶ and this is consistent with data from other parts of Africa.^{59,60} Furthermore, the clinical spectrum of heart disease within black South Africans

is broadened by cardiac complications related to HIV and tuberculosis.⁵⁶ The latter is not surprising, as South Africa is the country with the highest HIV infection rate in the world.⁶¹

2.2. Cardiovascular disease and HIV

Cardiovascular disease is multifactorial in etiology.⁶² Cardiovascular risk is determined by a complex interplay of several traditional cardiovascular risk factors, such as dyslipidemia, hypertension, diabetes, older age, cigarette smoking and positive family history.^{2,4,62} No meaningful difference is seen in the prevalence of hypertension between HIV infected and non-infected people,⁴ but cardiac involvement has been documented worldwide in various forms among HIV infected people.^{10,63} As treatment improves and people have a longer life expectancy, cardiovascular complications can be expected to be the cause of death in more and more cases. Dyslipidemia has been reported in HIV infected individuals with or without antiretroviral therapy,¹⁴ and it has also been reported that HIV infected people show an increased cardiovascular risk, even in the absence of the clustering of metabolic risk factors.⁵² Untreated HIV infection has also been associated with premature atherosclerosis²¹ and uncontrolled viremia may be more of a cardiovascular risk than controlled infection.²¹ It is, therefore, clear that the evaluation of the cardiovascular risk for people infected with HIV is determined by a complex interplay of direct and indirect vascular effects (vascular inflammation) of the infection itself,²¹ opportunistic infections, dyslipidemia associated with HIV^{3,8,20} the antiretroviral treatment, aging and exposure to classic cardiovascular risk factors.^{2,4,12,21} It is, therefore, understandable that Hsue *et al.*, reason that HIV infection itself could count as a risk factor, similar to traditional cardiovascular risk factors.¹⁰

2.3. Influence of HIV on the lipid profile

Dyslipidemia is characterized by an abnormal lipid profile, involving LDL-C, HDL-C and TG²³ and is likely to contribute to the elevated risk of CVD.^{20,23} HIV infection (with and without treatment) is associated with dyslipidemia, namely hypocholesterolemia with low levels of both LDL-C and HDL-C, as well as hypertriglyceridemia,^{3,6,10,14,15,20} and it remains unclear what the relative contribution of the infection itself, the treatment with antiretroviral therapy, and the interaction between the two to dyslipidemia and increased cardiovascular risk in HIV infected people is.^{2,20} The elevations in TG levels are mostly seen in the advanced stages of HIV infection, especially if the CD4 counts are below 200cells/mm³.⁶ However, researchers found that after initiation of HAART, levels of TG as well as LDL-C rose, though HDL-C remained below baseline.^{3,8,15}

The links between HIV infection and metabolic derangements or dyslipidemia are not clear¹⁴ and more research is needed to elucidate the underlying causes of the changes in the lipid profiles¹⁵ in order to direct appropriate interventions and minimise CVD risk. The effect of HIV on the HDL-C levels may be mediated by general or local inflammatory responses or other indirect effects of HIV on the liver.²⁰ Liver cells are responsible for forming most of the HDL-C particles in plasma and extrahepatic cells, including lymphocytes, contribute very little.²⁰ These liver cells are not targets for viral replication, but it is conceivable that a soluble protein secreted in the plasma by HIV may reduce cholesterol mobilization of these cell types and reduce the pool of HDL-C.²⁰ The high TG levels on the other hand may be the result of decreased TG clearance¹⁴ which Hsue *et al.*, found to correlate with higher interferon- α levels.¹⁰ Increased hepatic uptake of free fatty acids increases triglyceride synthesis and such increased uptake may in part further account for the hypertriglyceridemia.⁶⁴

2.4. HIV and lipodystrophy

Lipodystrophy is a general term describing varying degrees of fat redistribution,¹⁴ characterized by dyslipidemia, visceral adiposity, and loss of abdominal and peripheral subcutaneous fat.^{8,21} Lipodystrophy is increased in HIV infected people and is associated with an increased risk of developing CVD.⁸ HIV lipodystrophy is an insulin resistant, dyslipidemic state, and though it is mostly associated with the antiretroviral therapy,^{8,21} a small proportion of therapy naive HIV infected people may have lipodystrophy.⁸ The etiology of lipodystrophic changes in these therapy naive cases is not known, but these changes may occur as a result of an inflammatory response to the virus or as direct effects of the virus itself.⁸ According to Fisher *et al.*, multiple studies have shown that insulin resistance often precedes lipodystrophy, suggesting that insulin resistance may be a primary feature of the metabolic syndrome in the HIV infected population.³ HAART associated lipodystrophy shares similarities with the insulin resistant metabolic syndrome associated with CVD, but it is different disease processes, where peripheral fat wasting for example is not seen in metabolic syndrome associated with CVD.²¹ It is also not clear if HAART associated lipodystrophy carries the same risks as the metabolic syndrome²¹ associated with increased risk of CVD.⁸

According to Tershakovec *et al.*, it has been suggested that the risk of lipodystrophy may be associated with changes in immune status.¹⁵ The development of lipodystrophy could at least be partially mediated by cytokines since interferon- α and C-reactive protein (CRP) are increased in AIDS, and TG levels are associated with interferon- α levels in HIV infected

people.¹⁴ Lipodystrophy has also been associated with a lower expression of LDL-C receptors.¹⁵

3. Vascular dysfunction and HIV

3.1. Endothelium, vascular function and HIV

A healthy endothelium is smooth and relatively unsusceptible to the adherence of leukocytes and inflammatory mediators,⁶² and maintains vascular homeostasis by regulating vascular tone, smooth muscle cell proliferation, and thrombogenicity.⁶⁵ The endothelium is thus a regulatory organ that participates in immune responses and plays a critical role in the progression and outcome of infectious diseases, regulates blood pressure and is involved in the metabolism of lipoproteins.⁶⁶ As an interface between the blood and the vascular smooth muscle, the endothelium is constantly exposed to noxious circulating agents such as cholesterol, infective agents and cigarette by-products.¹² In HIV infection the endothelium is under the combined influence of a viral load, the adventitial cell activation by HIV, the effects of gp 120 and Tat, cytokines, opportunistic infections, increased concentration of circulating antigens and immune reconstruction.^{26,67} It is, therefore, clear that the endothelium of HIV infected people has a clustering of conditions that activate or injure the endothelium,²⁶ causing functional alterations of the endothelium that resemble inflammation as found during the development of atherosclerosis¹² and leads to endothelial dysfunction.

Endothelial dysfunction is characterized by an increased production of inflammatory cytokines and adhesion molecules, leading to leukocyte capture and migration into the endothelium⁶² and the normal endothelial phenotype of vasorelaxant, anticoagulant, antiplatelet, and profibrinolytic changes to one that is vasoconstrictive, procoagulant, platelet-activating and antifibrinolytic.⁹ It results in a dysbalance of vascular regulatory mechanisms, which causes damage to the arterial endothelium,⁶⁵ and finally the formation of a thrombus² which is a key event in cardiovascular disease.⁹ Endothelial dysfunction is, therefore, considered a key factor in atherosclerosis-related diseases and is present in all stages of atherosclerosis.⁹

Endothelial dysfunction can be indicated non-invasively by measuring biological markers that are present on the surface of endothelial cells or are expressed in response to several stimuli and have an important role in the process of leukocyte rolling, firm adhesion and transendothelial migration.⁶⁷ Soluble CAM's such as ICAM, VCAM, E-selectin, and molecules that increase during endothelial damage (von Willebrand factor (vWF) and thrombomodulin)^{2,9} are considered reliable biomarkers of vascular dysfunction and atherosclerosis development.^{23,67} This adds to the predictive value of classic risk factors for

CVD in asymptomatic individuals.^{23,67} De Gaetano *et al.*, report that endothelial dysfunction was associated with the progression and severity of HIV infection and that researchers found a positive correlation between TG and VCAM-1, ICAM-1 and thrombomodulin levels.² Endothelial dysfunction was also associated with low HDL-C levels in HIV infected people⁹ and the endothelium of HIV-1 infected individuals is characterized by increased numbers of adherent leukocytes as compared to non-infected individuals.⁴⁶

The markers important for the evaluation of the effect of HIV on vascular function will be discussed in more detail later in the literature overview.

3.2. Atherosclerosis, inflammation and HIV

Atherosclerosis is characterized by a complex multifactorial pathophysiology⁶⁸ of the arterial wall. Research on atherosclerosis as an inflammatory condition has moved the understanding of the pathophysiology away from the concept of cholesterol and smooth muscle cells clogging the arteries, towards an understanding that the interaction between cholesterol and inflammation forms complex lesions that are ripe for thrombosis.⁶² The classical definition of inflammation has been 'the response to tissue injury'.^{69,70} Injury, resulting in distressed cells, is the trigger, and inflammation the consequence.⁶⁹ The tissue injury that occurs when cells have been stressed by an insult of some kind, such as bacterial or viral infection, hyperglycaemia, dyslipidemia²³ often results in metabolic impairment and local expression of cytokines.⁶⁹ Atherogenesis, the formation of the lesion in the arterial intima, is also a response to injury, with lipoproteins or other risk factors, such as infection, as the injurious agents.⁷⁰

Atherosclerosis is thus a chronic disease of the arterial wall where both innate and adaptive immunoinflammatory mechanisms are involved.⁷¹ Inflammation, mediated by cytokines⁷² is implicated in the formation of early fatty streaks, the earliest visible lesion of atherosclerosis⁷¹ and precursor of atherosclerotic plaque.³ The fatty streak comprises an area of intimal thickening composed of macrophages distended by lipid droplets (known as foam cells), lymphocytes, and smooth muscle cells⁷¹ which migrate from the medial layer of the endothelium.⁷⁰ Fatty streaks are not clinically significant, but they are the precursors of more advanced lesions characterized by the accumulation of lipid-rich necrotic debris and smooth muscle cells.⁷⁰ These advanced fibrous lesions typically have a fibrous cap consisting of smooth muscle cells and extracellular matrix that encloses a lipid-rich necrotic core.⁷⁰ With the secretion of fibrous elements by the smooth muscle cells, these occlusive fibrous plaques develop and increase in size. Initially, the lesions grow towards the

adventitia until a critical point is reached after which they begin to expand outwards and encroach on the lumen of the artery.⁷⁰

Inflammation is implicated when the endothelium is activated during atherosclerosis and expresses chemokines and adhesion molecules leading to monocytes/lymphocyte recruitment and infiltration into the subendothelium.⁷¹ When leukocytes infiltrate or enter the endothelium, they augment the inflammatory process by excreting more cytokines.⁶² The adherence of monocytes to the endothelium and their migration into the intima of the arterial wall are facilitated by the presence of the expressed cellular adhesion molecules.⁷³ Monocytes undergo differentiation once trapped in the arterial wall. In the arterial intima, monocytes that differentiate into macrophages are characterized by a continuously increasing volume of cytoplasm containing vesicles, vacuoles, primary and secondary lysosomes.⁷³ The majority of monocytes, differentiated into macrophages, absorb lipids (for example modified LDL-C) in their cytoplasm, they become lipid-laden and this transform them into foam cells.^{23,62,73} Trapped LDL-C undergo modification in the vessel wall, including oxidation (as a result of exposure to oxidative waste of vascular cells), lipolysis, proteolysis and aggregation.⁷⁰ The rapid uptake of oxidized (and otherwise modified) LDL-C particles by macrophages, leading to foam cell formation, is mediated by so-called scavenger receptors which are regulated by cytokines such as TNF- α .⁷⁰ Aggregation of foam cells leads to plaque formation and the formation of an atheromatous core⁷³ by the excretion of various cytokines and some proliferation of smooth muscle cells.⁶²

Inflammation, macrophage infiltration, extracellular matrix digestion, oxidative stress, lipid deposition, calcification, cell apoptosis and thrombosis are among further molecular mechanisms contributing to plaque development and progression.⁶⁵ While the formation of atherosclerotic plaque is characterized by the accumulation of inflammatory cells and oxidized LDL-C that reduce the intracellular nitric oxide (NO) and activate endothelial cells,⁹ it is interesting to note that HIV-1 induces apoptosis of endothelial cells¹² where gp 120 and Tat interact with monocytes receptors to induce the apoptosis.⁵² As this process progresses, the atheromatous centres (cores) of plaque become necrotic, consisting of lipids, cholesterol crystals and cell debris.⁷³ In the central portion of the atheromatous core, the destruction of foam cells (probably by apoptosis) occurs and this is accompanied by the extracellular accumulation of lipids and cellular debris.⁷³ Inflammation also acts on the onset of adverse clinical vascular events, when monocytes-derived macrophages and activated endothelial cells secrete various cytokines, chemokines and growth factors.^{62,68,70,71} Activated cells within the plaque also secrete disintegrins like matrix proteases that degrade extracellular matrix proteins and weaken the fibrous cap, ultimately leading to rupture and thrombus

formation.^{62,68,70,71} This usually occurs at the site of the disrupted plaque, which is rich in foam cells, suggesting that the factors contributing to inflammation may also influence thrombosis^{62,70} which is the key event leading to myocardial infarction and stroke.^{3,70}

The higher rates of atherosclerosis seen in HIV-1 infected people, although controversial, are usually contributed to classic cardiovascular risk factors and to side effects of the antiretroviral therapy.⁴⁸ However, the chronic infection status of the HIV infected people that triggers the inflammatory response might also lead to a higher risk of atherosclerosis⁴⁸ as inflammation is central at all stages of atherosclerosis⁷¹ and chronic low-grade inflammation (as seen in HIV infections) is associated with accelerated atherosclerosis¹⁰ and damage of the endothelium.¹² Inflammation has been identified in the early risk period which includes endothelium dysfunction, expression of adhesion molecules, recruitment of leukocytes to the injured endothelium and migration of monocytes to the arterial intima.²³ Atherosclerosis in general does not progress rapidly, but develops over years and is usually asymptomatic in the early stages of the disease.²³

In an attempt to improve cardiovascular risk prediction, circulating molecules related to chronic inflammation have emerged as potential biomarkers for atherosclerosis.⁷⁴ Inflammatory markers, such as CRP and interleukins (such as IL-6), which have been demonstrated to have higher levels in HIV infection⁷² have strong and independent prognostic implications in patients with atherosclerotic vascular disease.¹² The activation of pro-inflammatory cytokines such as TNF- α or IL-6 increases the endothelial expression of adhesion molecules such as ICAM and VCAM that facilitate the migration of inflammatory cells and monocytes to the sub-endothelium.⁹ Lipids also play a role in the risk assessment, as the enzyme paraoxonase 1, located on HDL-C, has potent antioxidant properties by hydrolyzing oxidized lipids formed on LDL-C and HDL-C.^{70,72} This enzyme inhibits the oxidation of lipoproteins in the subendothelial space that, otherwise, could lead to the formation of foam cells.⁷² Besides inhibiting lipoprotein oxidation, HDL-C also plays a role in the removal of excess cholesterol from peripheral tissue and is, therefore, strongly protective against atherosclerosis.⁷⁰

3.3. Thrombotic events and HIV

Atherosclerotic cardiovascular events are commonly manifested via a thrombotic event²⁶ and thrombosis is responsible for clinically observable adverse effects, implicating coronary, cerebrovascular, and peripheral vascular beds.⁷¹ The process of clotting involves coagulation, limited and controlled anticoagulation, and the counterbalancing process of fibrinolysis, limited by antifibrinolysis.⁷⁵ One of the consequences of endothelial dysfunction

may include changes in the ability of the endothelium to participate adequately in both coagulation and fibrinolysis and may thus predispose to thrombosis.²⁶ During endothelial dysfunction thrombin production is activated on the surface of endothelial cells⁹ and fibrin is a consistent component of atherosclerotic plaques.⁷⁶ Increased expression of plasminogen activator inhibitor-1 (PAI-1) leads to alterations in fibrinolytic activity and this promote fibrin deposition and extracellular matrix accumulation in atherosclerotic lesions.⁷⁶

Intertwined with the immunological abnormalities in HIV infected persons are a wide range of coagulation disorders that may be associated with HIV infection itself.²⁷ Fibrinogen and CRP determination may be helpful screening tools to identify individuals at added risk for thrombotic complications of CVD.⁷⁵ CRP and fibrinogen were higher in HIV infected people¹⁰ and depending on the plasma HIV load, a hypercoagulable state is induced in HIV infected people¹² making this population more susceptible to thrombotic events. Cigarette smoking may augment the enhanced platelet activation seen in HIV infected persons.²⁷ Smoking also reduces HDL-C cholesterol levels, but Hsue *et al.*, found no difference in the HDL-C levels of smoking and non-smoking HIV infected individuals.¹⁰

4. Biological markers associated with HIV

Biological markers are important research tools⁶⁸ used in non-invasive models to study endothelial function.⁶⁷ Biological markers associated with vascular function are present on the surface of endothelial cells or are expressed in response to several stimuli.⁶⁷ Measurement of serum biological markers may supply important prognostic and/or diagnostic information indicating non-traditional risk, thus information independent of traditional risk factors.^{26,68} Investigators may gain much information by measuring biological markers, but have to keep in mind that monitoring only one or a few markers may lead to erroneous conclusions.²³

4.1. Pro-inflammatory markers

4.1.1. C-Reactive Protein (CRP)

CRP is an acute phase protein,²⁶ a reliable and accessible marker of inflammation^{26,72,71} and synthesized by hepatocytes upon stimulation by several cytokines primarily IL-6 and also TNF- α .^{26,68,71} CRP is a robust clinical marker because of its analytic stability, shows reproducible results and is biologically stable over time.^{62,68} CRP can be increased to about 1000 fold in response to inflammation, but lower levels, implicated in increased cardiovascular risk, are detected by a high-sensitivity assay.^{62,71} However, minor elevation of CRP (3-10 mg/L) is also associated with minimal environmental irritants, a variety of

dietary patterns and apparently non-inflammation conditions, many of which imply mild degrees of tissue injuries.⁶⁹

CRP may also be produced locally in vascular smooth muscle cells and macrophages in atherosclerotic lesions and through its direct proinflammatory effects contribute to the initiation and progression of atherosclerotic lesions.⁶⁸ CRP has been shown to enhance the expression of ICAM-1, VCAM-1, and monocyte chemoattractant protein (MCP), may stimulate PAI-1^{62,71} and have prognostic implications in persons with atherosclerotic disease.¹² Verma *et al.*, suggest that CRP has several effects that may influence progression of vascular disease, including activation and chemoattraction of circulating monocytes, mediation of endothelial dysfunction, induction of a prothrombotic state, increase of cytokine release and activation of the complement system,⁷⁷ but according to Koenig and Khuseyinova, there is conflicting evidence and it should be interpreted with caution.⁶⁸ Research of the past decade provides strong evidence for CRP to predict future cardiovascular (CV) risk,⁶⁸ and for high sensitivity CRP to predict CVD independent of the presence of traditional risk factors such as hypertension, smoking, diabetes, age, and HDL-C levels.⁶² Masia *et al.*, suggest that CRP concentration above 0.3 mg/dl may be considered a high risk for developing cardiovascular complications⁷⁸ and high CRP is associated with an increase in the prevalence of myocardial infarction, stroke and peripheral vascular disease.⁶²

Whether CRP is also a marker of cardiovascular risk in HIV infected persons remains, according to Masia *et al.*, unknown.⁷⁸ CRP is increased in HIV positive persons^{10,52} probably related to metabolic abnormalities and altered fat distribution.^{8,52} It has been associated with the prognosis of HIV infection, but no correlation was found between CRP levels and HIV viral load or CD4 cell counts.⁷⁸ Van Wijk *et al.*, reason that elevated CRP levels is most likely because of the chronic immune activation associated with the HIV infection.⁵²

4.1.2. Interleukin 6 (IL-6)

IL-6 is a 26-kDa single chain glycoprotein produced by many cell types including activated monocytes/macrophages and endothelial cells.⁶⁸ It can be generated from central fat and is a major cause of endothelium dysfunction.²³ It is a proinflammatory cytokine, thus an early stimulator of the inflammatory process⁶² and stimulates the synthesis of CRP and fibrinogen by hepatocytes.^{23,71} Endothelial cells stimulated by IL-6 express ICAM-1.⁶⁸ Although the most important function of IL-6 is the amplification of the inflammatory cascade, it represents the principal procoagulant cytokine, induces a prothrombotic state and has important direct proatherogenic properties.⁶⁸ People infected with HIV have higher levels of IL-6.³

4.1.3. Soluble urokinase plasminogen activator receptor (suPAR)

SuPAR (NCBI Accession no. AAK31795) is the soluble form of the urokinase-type plasminogen activator receptor (uPAR) which is a glycosyl-phosphatidylinositol (GPI)-linked membrane protein.⁷⁹ The calculated mass of the soluble protein is 60 kDa.⁷⁹ uPAR is expressed on monocytes, macrophages, activated T-lymphocytes, natural killer cells and neutrophils.⁸⁰ Soluble uPAR originates from cleavage and release of the membrane-bound uPAR and is present in plasma, urine, blood, serum and cerebrospinal fluid.⁸¹ uPAR mediates a variety of functions in terms of vascular homeostasis, inflammation and tissue repair.⁸²

Conclusions from studies suggest suPAR to be involved in numerous physiological pathways, including the plasminogen activating pathway, inflammation, modulation of cell adhesion, migration and proliferation.⁸¹ In healthy individuals, suPAR levels are quite stable, do not show major variation in blood and circadian suPAR levels have been shown to be constant.⁸³ Increased activation of the immune system leads to increased serum suPAR levels in several pathological conditions including HIV-1 infection,⁸¹ increased suPAR levels are prognostic of poor outcome in infectious diseases,⁸⁴ and are positively correlated with pro-inflammatory biomarkers such as TNF- α , leukocyte count⁸⁵ and with CRP levels.⁸⁶

The uPA-mediated conversion of plasminogen to plasmin, occurring at the leading edge of cells, promotes cleavage of fibrinogen to fibrin and degradation of other extracellular membrane proteins.⁸⁷ The coagulation pathway and uPAR system have been related to CVD and atherosclerosis, and the presence of CVD was predicted by suPAR in haemodialysis patients, thus the urokinase-mediated fibrinolysis may contribute to the increased risk of cardiovascular disease in this population.⁸⁸ The suPAR system is involved in atherosclerosis and intima-media thickness (IMT) was independently related to high suPAR in uraemic patients.⁸⁹ Soluble uPAR can be released into the circulation from monocytes, vascular endothelial and smooth muscle cells at sites of vascular pathologies such as in atherosclerotic plaque, where suPAR may become upregulated.⁸⁸

Soluble uPAR may reflect the metabolic status of HIV infected patients on HAART, thus linking low-grade inflammation, immune constitution, lipid and glucose metabolism, and fat redistribution.⁸³

4.2. Markers of vascular function

4.2.1. Vascular- and Intracellular Adhesion Molecules (VCAM-1 and ICAM-1)

VCAM-1 and ICAM-1 belong to the immunoglobulin family.² These molecules are endothelial ligands for integrins expressed on leukocytes and platelets.^{3,9} Upon activation by pro-inflammatory cytokines such as IL-6 they facilitate endothelial adhesion and the migration of inflammatory cells and monocytes to the sub-endothelium.⁹ An increased expression of VCAM-1 and ICAM-1 is thus associated with an increased intimal leukocyte accumulation and the abundance of adhesion molecules on arterial sites prone to the development of atherosclerotic lesion.^{9,23,73} Adhesion molecules may be seen as reliable biological markers for atherosclerosis² and add to the predictive value of classic risk factors for coronary artery disease.⁶⁷

Higher plasma levels of VCAM-1 and ICAM-1 are seen in HIV infected people^{2,3,90} and HIV Tat protein induces these adhesion molecules and endothelial cell adhesion in cultured vascular endothelial cells.³ According to Galea *et al.*, the higher levels of VCAM-1 and ICAM-1 do not simply reflect a general pattern of inflammation, but are specifically regulated and are dependent on the clinical status of infected people.⁹⁰ ICAM and VCAM can also be influenced by statins, smoking, obesity and ethnicity as lower levels of VCAM and ICAM are seen in people from African origin.⁹

4.2.2. Pulse wave velocity as measurement of arterial stiffness

The development of atherosclerosis shows no clinical symptoms and can develop for several years before detected.²³ If a thrombotic event does not occur and as atherosclerosis progresses to an advanced stage, the blood vessel will become harder and narrower and this change in the properties of the vessel will lead to symptoms such as arterial stiffness or reduced compliance.²³ The viscoelastic properties of large arteries can be described in terms of compliance, distensibility, or stiffness of the aorta or an individual artery.⁹¹ The above variables are usually determined from systolic-diastolic changes in diameter coupled with measurements of the local pulse pressure (PP).⁹¹ PP is influenced by left ventricular ejection (stroke volume and ejection time) and arterial stiffness.⁹¹ PP can thus be described as the pressure pulse generated by ventricular ejection, that is propagated throughout the arterial tree at a speed which is determined by the elastic and geometric properties of the arterial wall (its thickness and the arterial lumen diameter) and the characteristics (density) of the blood.⁹² PP is a marker of CV risk in the general population, particularly in older individuals with increased aortic stiffness as the predominant cause of increased PP.⁹³ Arterial stiffness, such as with age, increases PP by reducing compliance and increasing

pulse wave velocity (PWV),⁹⁴ a marker of arterial stiffness⁹¹ and an independent predictor of CV risk.⁹³ By the measure of PWV arterial stiffness can be more directly evaluated.⁹¹

Compliance is the absolute change in area for given change in pressure and serves a capacitive function in the large conduit arteries which store blood during systole.⁹⁵ During systole the contraction of the left ventricle myocardium and the ejection of blood into the ascending aorta dilate the aortic wall and generate a pulse wave which propagates along the arterial tree at a finite speed.⁹² This propagation velocity constitutes an index of arterial distensibility and stiffness: the higher the velocity, the higher the rigidity of the vascular wall and the lower the distensibility.⁹² Aortic PWV is a non-invasive measurement of arterial stiffness that was first reported in 1922.⁹⁶ PWV is now recognized as being a highly sensitive measurement of arterial tree stiffness,⁹⁶ is associated with atherosclerotic pathologies and arterial intima-media thickening, and is an independent predictor of cardiovascular mortality.^{52,96}

There is an increased risk for HIV infected persons to develop atherosclerosis.^{10,72} It is, therefore, increasingly important to identify those HIV infected people who have the highest risk for atherosclerosis, in such cases PWV may be used to investigate arterial stiffness⁵² and the development of atherosclerosis.

4.2.3. Carotid intima-media thickness

Other instruments such as ultrasound may also be helpful to differentiate between atherosclerosis and early risk.²³ Van Wijk *et al.*, found PWV and carotid intima-media thickness (IMT) to be related to variables of HIV infection, and time on antiretroviral therapy.⁵² Hsue *et al.*, on the other hand, found that IMT is increased in HIV positive subjects, is associated with classic coronary risk factors and with a nadir CD4 cell count of ≤ 200 cells/mm³,¹⁰ suggesting that immunodeficiency and traditional coronary risk factors may contribute to atherosclerosis.

4.3. Pro-thrombotic factors

Haemostatic factors associated with the development of CVD include fibrinogen and PAI-1, amongst others, and most haemostatic factors are intercorrelated with inflammatory markers such as CRP and also with LDL-C.⁷⁵

4.3.1. Fibrinogen

Fibrinogen is an acute phase protein,⁷⁵ a systemic inflammation marker relating to the clotting system,²³ and it may be seen as a coagulation marker.⁶² Fibrinogen is synthesized

by hepatocytes through the stimulation of IL-6.²³ Fibrinogen is a haemostatic risk factor for CVD,^{23,75} because of its role in platelet aggregation, plasma viscosity and fibrin formation.⁷⁵ Fibrinogen mediates the thrombogenic effect of other risk factors, increases with the number of cigarettes smoked and the correlation between fibrinogen and LDL cholesterol suggests a lipid-imposed CVD risk.⁷⁵ Thus Kannel concluded that fibrinogen and CRP determination may be useful screening tools to identify individuals at risk for thrombotic complications.⁷⁵ Hsue *et al.*, found fibrinogen to be increased in HIV positive people.¹⁰

4.3.2. Plasminogen activator inhibitor-1 (PAI-1)

PAI-1 is a 50 kDa plasma glycoprotein, a member of the serpin family⁹⁷ and linked to inflammatory and prothrombotic markers.⁷⁶ A variety of cells produce PAI-1 including hepatocytes, vascular smooth muscle cells, fibroblasts, mesangial cells and adipocytes.^{97,98} Several growth factors, a variety of cytokines e.g. TNF- α and IL-6, as well as metabolic products such as TG, free fatty acids and glucose stimulate PAI-1 expression.^{76,98} The synthesis of PAI-1 is increased in activated or injured endothelial and smooth muscle cells, and PAI-1 is also secreted by activated platelets.⁷⁶ PAI-1 promotes clot formation by controlling fibrinolysis as it inhibits the conversion of inactive plasminogen to active plasmin, a fibrin degrading protease, by binding tissue- and urokinase type plasminogen activators.^{97,98} PAI-1 is seen as a coagulation marker,⁶² and elevated levels are associated with endothelial dysfunction, CVD, diabetes⁹⁸ and metabolic syndrome.⁹⁷ High plasma levels of PAI-1 may also be associated with the development of atherosclerosis⁷⁶ where it promotes atherosclerotic plaque formation by decreasing the capacity to degrade fibrin, thus enhancing the chance for a damaging thrombosis to develop on plaque rupture.⁹⁸

PAI-1 reveals a circadian rhythm, and levels peak in the early morning corresponding with a nadir in net fibrinolytic activity.⁹⁹ PAI-1 plasma antigen levels vary between 15-30 ng/ml in healthy adults,⁹⁹ but measurement may be affected by PAI-1 release from platelets collected as part of the blood sampling.⁶² In HIV infected people increased levels of PAI-1 are associated with lipodystrophy and HAART.^{27,100}

MOTIVATION FOR THE STUDY

The heavy burden of Southern Africa's communicable diseases like HIV and tuberculosis is paralleled by a growing threat of non-communicable diseases.⁵⁴ Non-communicable diseases, such as cardiovascular disease, type 2 diabetes, cancer, chronic lung disease and depression, are emerging in both rural and urban populations.⁵⁵ South Africa is the country with the highest HIV infection rate in the world,⁶¹ and the overwhelming prevalence of HIV and AIDS masks the burden of the non-communicable diseases which is predicted to

increase significantly over the next decades.⁵⁵ Antiretroviral therapy was introduced in the late 1990's¹⁰¹ in industrialized countries such as Europe and North America where HIV-1 subtype B prevails,^{28,102} and reduced the morbidity and mortality associated with HIV and AIDS. In South Africa, the ART roll-out programme was introduced in February 2004 giving HIV infected individuals access for the first time to free ART.³³ This led to a decline in the HIV related mortality.¹⁰³

HIV infection paradoxically affects cardiovascular risk factors and circulatory disease within populations and individuals. HIV infection and especially the use of ART increases the risk for cardiovascular disease¹⁰⁴ and are associated with an increase in insulin resistance, dyslipidemia,⁵ lipodystrophy,^{7,16} endothelial dysfunction,⁹ accelerated atherosclerosis¹⁰ and coagulatory disorders.²⁷ These complications could become a serious health problem in South Africa by increasing the burden of non-communicable diseases further once patients are receiving ART for longer periods.⁵⁵

There is an abundance of literature regarding research on HIV-1 infection and cardiovascular influences of westernised Caucasian populations, but literature concerning the HIV-1 infected black population of South Africa, especially longitudinal data, is lacking to a great extent. Not only does the HIV-1 subtype prevalent in South Africa differ genetically from subtype B, prevalent where most of the research was done, but the black South African population has unique characteristics, living conditions and cultural backgrounds – which makes extrapolation from Caucasian populations difficult. Although the clinical consequences of the subtype variations still remain unclear,³⁰ no study to date has been done to evaluate the influence of HIV-1 (subtype C) infection itself, and the treatment thereof, on the vascular function and cardiovascular risk of the HIV-1 infected population of South Africa. In South Africa there is still a lack of knowledge regarding HIV infection and probably due to this lack of knowledge, the illness itself and poverty,¹⁰⁵ stigmatization,^{105,106} and/or skepticism towards the conventional treatment of HIV,³³ many HIV infected individuals choose not to enroll for treatment. This enabled the study of the effect of the infection itself as well as the effect of the treatment (over a three year period) in this unique population group.

In most epidemiological studies for evidence of cardiovascular disease in HIV infected patients, the choice of an appropriate control group with similar demographic background to that of the infected group is difficult and may limit comparisons in such studies.¹⁰⁷ The participants of this study were from the same locality, therefore, environmental factors and region-specific factors which could contribute to differences in lipid and metabolic

complications between the HIV infected and control participants could not have influenced the results.

Uncertainties and controversies in the literature

There are still many uncertainties regarding HIV-1 infection and cardiovascular risk in the literature which also played a role in the motivation for the study:

- The involvement of HIV infection in cardiovascular risk can be associated with various factors, such as the infection itself, opportunistic infections, the therapy or non HIV related cardiovascular risk factors like smoking and age.¹²
- It remains unclear what the relative contribution of the HIV infection and the ongoing inflammation are to the blood lipids.^{2,30}
- The role of HIV infection as a risk factor for premature atherosclerosis is still controversial.^{48,108}
- The influence of race/ethnicity on blood lipids, and thus metabolic and cardiovascular complications of the treated HIV-1 infected population is uncertain.¹⁰⁹

Motivation for each manuscript in the thesis

This thesis consists of three manuscripts submitted for publication. For the first two manuscripts data of the baseline (2005) study were used, while the baseline and follow-up (2008) data of the 294 participants who participated in the follow-up study were used for the third manuscript (Fig. 1).

In 2005 the participants were unaware of their HIV-1 infected status and, therefore, have not received antiretroviral treatment before. A case-control design was applied in the studies for the first two manuscripts and the control participants were matched according to age, gender, body mass index (BMI) and locality with the HIV-1 infected participants.

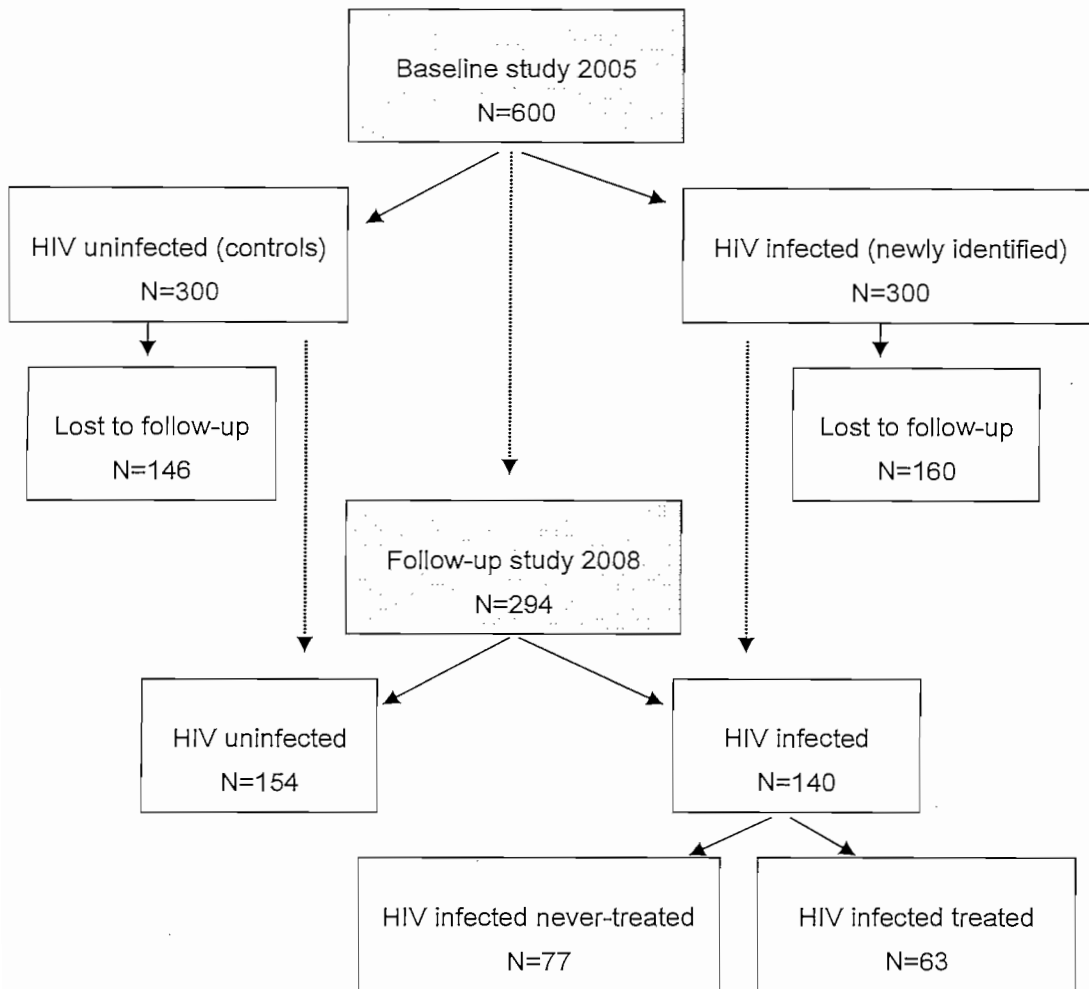


Figure 1: Outline of the study.

A decline was seen in the HIV related mortality,¹⁰³ after the introduction of the antiretroviral roll-out programme in February 2004. By enrolling to the antiretroviral roll-out programme HIV infected individuals had access to free antiretroviral therapy for the first time.³³ However, the effect of the treatment on the cardiovascular and metabolic status of the South African HIV-1 infected population still needs to be determined. The participants of the follow-up study (third manuscript) were HIV-1, most likely subtype C infected for at least 3 years. Sixty-three participants enrolled in the community-based roll-out programme and received treatment (Fig. 1). Thus the influence of both the treatment and the infection could be assessed.

Relevant literature background for each aspect of the study is discussed in the articles and a brief motivation, and an aim and hypotheses for each chosen topic will be provided before each manuscript.

AIM OF THE STUDY

Due to the numerous uncertainties regarding the contribution of HIV-1 infection to cardiovascular disease, the main aim of this study was to assess the influence of the HIV-1 (most likely subtype C) infection and the first-line antiretroviral therapy on vascular function in black Africans of the North West Province of South Africa.

HYPOTHESES

In view of the aim and the literature study the following hypotheses were formulated:

- No differences exist in cardiovascular variables (blood pressure, pulse pressure and mean arterial pressure (MAP) between HIV infected and uninfected participants. Antiretroviral treatment is associated with increases in blood pressure.
- HIV infection itself influences blood lipids and is associated with an abnormal lipid profile characterized by low levels of HDL-C and high TG levels. Inflammation is increased in the HIV-1 infected population.
- Participants on the World Health Organisation's recommended first-line therapy show an increase in HDL-C levels and a decrease in triglyceride levels.
- HIV-1 infection, and the inflammation it induces, are associated with endothelial dysfunction, accelerated atherosclerosis and increased coagulation in black South Africans.
- SuPAR levels are increased in HIV-1 infected participants and decreased in the treated participants.
- HIV-1 infection itself, or the treatment thereof, or both, may influence the vascular function of HIV-1 (most likely subtype C) infected black South Africans.

STRUCTURE OF THE THESIS

This thesis consists of three manuscripts submitted for publication. Following this introductory chapter (Chapter 1), the first manuscript (Chapter 2) compares the lipid profiles of the never-treated HIV-1 subtype C infected and uninfected Africans. The second manuscript (Chapter 3) investigates the association between inflammation and endothelial dysfunction in the never-treated HIV-1 infected participants, while the third manuscript (Chapter 4) investigates the influence of the World Health Organisations (WHO's) recommended first-line therapy after three years on the HIV-1 infected participants. In Chapter 5 a summary and discussion of all the results are given, conclusions are drawn and recommendations made. The relevant references are provided at the end of each chapter according to the author's instructions for the specific journal in which the article was published or submitted for publication. To keep the reference style uniform throughout the

thesis, the relevant references in the unpublished Chapters 1 and 5 are also according to the most used journal style, namely the Vancouver style. This is according to the specifications of the North-West University.

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CHAPTER 2

LIPID ABNORMALITIES IN A NEVER TREATED HIV-1 SUBTYPE C INFECTED AFRICAN POPULATION

LIPID ABNORMALITIES IN A NEVER TREATED HIV-1 SUBTYPE C INFECTED AFRICAN POPULATION

MOTIVATION FOR THE MANUSCRIPT

HIV infection (with and without treatment) influences the lipid profile and is associated with dyslipidemia, characterised by hypercholesterolemia with low levels of TC, LDL-C and HDL-C and hypertriglyceridemia (1-6). Antiretroviral therapy is mostly associated with lipodystrophy (fat redistribution), which is increased in HIV infected individuals (7). However, lipodystrophy is also seen in a small proportion of HIV infected people who are therapy naive (8). Dyslipidemia and lipodystrophy are reminiscent of the metabolic syndrome (8), which is mostly seen in treated HIV-1 infected individuals (8), but was also reported in untreated individuals (9). The above may contribute to an elevated risk of cardiovascular disease (3,8,10). The influence of HIV infection and the ongoing inflammation on the blood lipids and the development of lipodystrophy remains unclear (11,12) and motivated this study.

In 2005, during the baseline study, the participants of the study were newly identified as being HIV-1 infected and have never received antiretroviral therapy. Therefore, the effect of treatment could be excluded for this part of the study. South Africa, where HIV-1 subtype C prevails, is the country with the highest HIV infection rate in the world (13), while HIV-1 subtype B (genetically different from subtype C) prevails in Europe and the United States (14,15). Most of the research regarding HIV-1 infection has been done in the United States and Europe. There is still much uncertainty about the clinical consequences of subtype variations and the effect of the HIV-1 subtype C virus on the cardiovascular system.

It was, therefore, decided to evaluate the lipid and anthropometric profile and prevalence of the metabolic syndrome of the newly identified HIV-1 subtype C infected participants which may increase the risk for cardiovascular disease in these individuals. An increase risk for cardiovascular disease in HIV-1 infected individuals could have a major influence on the health system of South Africa, as antiretroviral treatment is not widely available (16).

AIM OF THE MANUSCRIPT

The aim of this study was to evaluate if HIV-1 (subtype C) infection itself is associated with dyslipidemia, inflammation and the occurrence of the metabolic syndrome in newly identified HIV infected participants who have never received antiretroviral therapy.

HYPOTHESES OF THE MANUSCRIPT

- Dyslipidemia and inflammation are detected in the HIV-1 infected participants compared to their uninfected controls.
- No increase in the metabolic syndrome prevalence is seen in never-treated HIV-1 subtype C infected participants.

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LIPID ABNORMALITIES IN A NEVER TREATED HIV-1 SUBTYPE C INFECTED AFRICAN POPULATION

Running title: HIV-1 and lipid abnormalities in Africans

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ABSTRACT

Dyslipidemia has been documented worldwide among human immunodeficiency virus (HIV) infected individuals and these changes are reminiscent with the metabolic syndrome (MetS). In South Africa, with the highest HIV infections worldwide, HIV-1 subtype C is prevalent, while HIV-1 subtype B (genetically different from C) prevails in Europe and the United States. We aimed to evaluate if HIV infection (subtype C) is associated with dyslipidemia, inflammation and the occurrence of the metabolic syndrome in Africans. Three hundred newly diagnosed HIV infected participants were compared to three hundred age, gender, body mass index and locality matched uninfected controls. MetS was defined according to the Adult Treatment Panel III (ATP III) and International Diabetes Federation (IDF) criteria. The HIV infected group showed lower high density lipoprotein cholesterol (1.23 vs. 1.70 mmol/L) and low density lipoprotein cholesterol (2.60 vs. 2.80 mmol/L) and higher triglycerides (1.29 vs. 1.15 mmol/L), C-reactive protein (3.31 vs. 2.13 mg/L) and interleukin 6 (4.70 vs. 3.72 pg/L) levels compared to the uninfected group. No difference in the prevalence of the MetS was seen between the two groups (ATP III – 15.2 vs. 11.5%; IDF – 21.1 vs. 22.6%). This study shows that HIV-1 subtype C is associated with dyslipidemia, but not with a higher incidence of MetS in never antiretroviral treated HIV infected Africans.

Keywords: HIV-1 subtype C, South Africa, African, metabolic syndrome, dyslipidemia, never-treated, inflammation.

INTRODUCTION

Southern Africa is accounted for almost a third of all new human immunodeficiency virus (HIV) infections and acquired immunodeficiency syndrome (AIDS) related deaths worldwide, and an estimated 5.5 million people are living with HIV in South Africa (1). The predominant virus responsible for these infections in South Africa is HIV-1, group M (major), subtype C (2,3), which accounts for 55-60% of all HIV-1 infections worldwide (4,5), and differs as much as 30% in its genomes from HIV-1 subtype B, responsible for the infections in North America, Europe and Australia (2, 5,6). The clinical consequences of these subtype variations still remain unclear (7).

Cardiovascular involvement in various forms, such as dyslipidemia (8), lipodystrophy (9,10), endothelial dysfunction (11), accelerated atherosclerosis (12) and coagulation disorders (13) have been documented worldwide among HIV infected individuals. This involvement can be associated with various factors, such as the infection itself, opportunistic infections, the therapy or non HIV related cardiovascular risk factors like smoking and age (14). It is well known that the lipid profile of HIV infected individuals changes, and these changes include increased levels of triglycerides (TG), and decreased levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) (12,15-18). It remains unclear what the relative contribution of the HIV infection and the ongoing inflammation are to the blood lipids (7,19).

Some of the changes seen in HIV infected individuals – in particular low HDL-C levels, hypertriglyceridemia, increased levels of visceral adipose tissue, plasma glucose and insulin – are reminiscent of the metabolic syndrome (MetS) (10). The MetS has been identified as a significant and multifaceted risk factor for cardiovascular disease (CVD) (10,20). The above metabolic abnormalities are among the most significant side effects experienced with highly active antiretroviral therapy, in particular with protease inhibitors (10,14,21). On the other hand, the long term effects of the infection itself (as antiretroviral therapy cannot completely eradicate HIV-1) have become increasingly challenging (22) and may play a role in these metabolic changes. This is seen in the study of Bonfanti *et al.* where the prevalence of the MetS was higher in the HIV infected individuals compared to the general population, but was similar in treated and untreated HIV infected individuals (23).

Even though South Africa is the country with the largest number of HIV infections in the world (1), to the best of the authors's knowledge, no study to date has been done to evaluate whether the HIV-1 (subtype C) infection itself, prevalent in South Africa (3), leads to the

same metabolic changes and inflammatory state seen in HIV-1 (subtype B), prevalent in Europe, United States, Australia and South America (5).

The aim of this study was, therefore, to evaluate if HIV-1 (subtype C) infection itself is associated with dyslipidemia, inflammation and the occurrence of the metabolic syndrome in newly identified HIV infected participants who have never received antiretroviral (ARV) therapy.

METHODS

Study design and participants

This sub-study is embedded in the larger international PURE (Prospective Urban and Rural Epidemiology) study. The overarching PURE study is a longitudinal multi-national study that will track changes in lifestyles, CVD risk factors and chronic diseases over a period of twelve years, using periodic standardized data collection in urban and rural areas of developing countries in transition, including South Africa. The South African leg of the study was performed in the North West Province where a total of 2000 black South Africans (1000 urban and 1000 rural) were randomly recruited from a rural and urban setting and screened during the baseline phase in 2005. The inclusion criteria were volunteers older than 35 years that were non users of any chronic medication and with no self reported diseases. For this sub-study the 300 newly identified HIV infected participants of the baseline PURE study population were individually matched with 300 HIV uninfected participants (case-control design), according to age, gender, body mass index (BMI) and locality (urban and rural). The methodology appropriate to this sub-study will be discussed.

Ethical considerations

All participants provided signed informed consent after all procedures were explained to them in their home language. The study protocol complies with the Declaration of Helsinki as revised in 2004 (24) and was approved by the Ethics Committee of the North-West University, Potchefstroom, South Africa.

Experimental protocol

Permission to execute the PURE study was obtained from the provincial Department of Health, local authorities and from the Tribal Chief in the rural area. For about twelve weeks 30-35 participants arrived at the research locality of the rural or urban areas at about 07:00 each morning after a 10-15 minute drive (provided by the research team) from their communities. The participants were introduced to the setup and after the procedures were explained they signed the informed consent forms and received HIV pre-counseling given by

trained counselors. The HIV status of the participants was revealed during post-counseling and the infected participants were referred to their local clinic or hospital for follow-up and CD4 cell count determination. During the course of the morning demographic, lifestyle and food frequency questionnaires were completed with the help of the specially trained field workers in the subject's home language. Lifestyle data included self reported current tobacco use, alcohol intake as well as medical history.

Anthropometric measurements

Height, weight, hip and waist circumference (WC) were measured (Precision Health Scale, A & D Company, Japan; Invicta Stadiometer, IP 1465, UK; Holtain unstretchable metal tape) using standardized procedures (25).

Cardiovascular measurements

Systolic blood pressure, diastolic blood pressure and heart rate were obtained with the validated OMRON HEM-757 device. After a 10 minute rest period blood pressure measurements were performed twice (5 minutes apart) on the right arm (brachial artery), while the participants were seated upright and relaxed with his/her right arm supported at heart level. Appropriate cuffs were used for obese participants.

Blood, serum and plasma samples

Blood was drawn from the antebachial vein using a sterile winged infusion set and syringes. Serum was prepared according to appropriate methods and stored at -80°C in the laboratory. In the rural area serum was stored at -18°C (no longer than five days) until it could be transported to the laboratory facility and was then stored at -80°C until analysis.

Biochemical analyses

Quantitative determination of the cholesterol, HDL-C, TG, glucose (GOD-POD), high sensitivity C-reactive protein (hs-CRP) and creatinine concentration in the serum of the participants was done with the Konelab20i™ auto analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland), a clinical chemistry analyzer for colorimetric, immunoturbidimetric and ion-selective electrode measurements. LDL-C was calculated by using the Friedewald formula (26). Creatinine clearance rate was estimated using the Cockcroft-Gault formula (27). Serum concentrations of high sensitivity interleukin-6 (hs-IL-6) were measured using human enzyme-linked immunosorbent assays (Quantikine® HS ELISA, R&D Systems, Minneapolis, USA). HIV status was determined with the First Response (PMC Medical, India) rapid HIV card test using whole blood. If tested positive, the test was repeated with the Pareeshak (BHAT Bio-tech India) card test. The card test distinguishes between HIV-1 and HIV-2, but

not between subtypes. The HIV-1 subtype C epidemic prevalent in South Africa has been established by serotyping and genotyping (1,2,3).

The metabolic syndrome

The MetS in the sub-study was identified according to the definition of the Third Report of the National Education Programme Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (ATP III) (20), and according to the definition of the International Diabetes Federation (IDF) (28).

Statistical analysis

All data were statistically analyzed by means of Statistica v.8 (Statsoft Inc., OK, USA, 2008). Mean values and standard deviations were calculated. The distribution of hs-CRP, CRP:HDL ratio and hs-IL-6 were normalised by logarithmic transformation before analysis, reporting the geometric mean and 5-95% percentiles. Independent T-tests were used to compare the characteristics of the continuous variables of the HIV infected and uninfected groups. The Chi-square tests were done to compare data of categorical variables. An analysis of covariance (ANCOVA) was performed to compare the cardiovascular parameters, lipid profile, glucose, hs-CRP and hs-IL-6 of the HIV infected and uninfected participants, whilst adjusting for tobacco and alcohol use.

Odds ratios (OR), as estimates of risk, with 95% confidence intervals (CI), were calculated using 2x2 frequency tables for HIV infected versus uninfected participants. Since very few participants met the cut-off values for the MetS (for both the ATP III and IDF definitions), especially for WC in African men, the median was used as cut-off value for the calculation of odds ratios. Frequency tables were used to determine the number of subjects that were classified according to each specific MetS definition (ATP III and IDF), thereby determining the prevalence of the MetS in both the HIV infected and control groups. Chi-square test was used to obtain the p values. P values ≤ 0.05 are regarded as significant.

RESULTS

Characteristics of HIV infected participants and matching controls are reported in Table 1. Due to individual matching, age and BMI values, as well as the other anthropometric measurements were almost identical in the two groups. HIV infected participants had a lower systolic blood pressure (SBP), pulse pressure, TC, HDL-C and LDL-C, whereas the heart rate, TG, TG:HDL-C ratio, hs-CRP, CRP:HDL-C ratio and hs-IL-6 were higher in the HIV infected group. After adjusting for tobacco and alcohol use, similar results were obtained, which were expected as the percentage tobacco and alcohol users did not differ.

There were no differences in the calorie and fat intake between the HIV infected and uninfected groups. Separate analyses as in Table 1 were performed for each gender and similar results were obtained.

Table 1

Characteristics of the HIV infected and uninfected African participants (N = 600).

	HIV infected (N = 300)	HIV uninfected (N = 300)	P
Age (years)	44.0 ± 8.04	44.0 ± 7.81	0.97
Height (cm)	161 ± 9.88	161 ± 8.37	0.62
Weight (kg)	59.5 ± 13.5	58.9 ± 13.9	0.61
BMI (kg/m ²)	22.9 ± 5.59	22.8 ± 5.48	0.92
Waist (cm)	75.9 ± 10.6	75.9 ± 10.1	0.98
Hip (cm)	92.6 ± 13.8	93.0 ± 13.9	0.71
Waist:hip ratio	0.83 ± 0.11	0.82 ± 0.10	0.62
SBP (mm Hg)	124 ± 21.8	129 ± 21.8	<0.01
DBP (mm Hg)	84.0 ± 14.7	85.9 ± 14.3	0.09
PP (mm Hg)	40.2 ± 11.5	43.7 ± 13.1	<0.01
HR (beats/min.)	76.4 ± 15.0	72.2 ± 15.2	<0.01
TC (mmol/L)	4.42 ± 1.25	5.02 ± 1.33	<0.01
HDL-C (mmol/L)	1.23 ± 0.58	1.70 ± 0.71	<0.01
LDL-C (mmol/L)	2.60 ± 1.01	2.80 ± 1.14	0.01
TG (mmol/L)	1.29 ± 0.77	1.15 ± 0.75	0.03
TG:HDL-C ratio	1.41 ± 1.47	0.86 ± 1.21	<0.01
Glucose (mmol/L)	5.35 ± 1.26	5.50 ± 1.10	0.13
hs-CRP (mg/L)	3.31 (0.32-50.4)	2.13 (0.23-29.2)	<0.01
CRP:HDL-C ratio	2.94 (0.21-62.0)	1.36 (0.13-18.5)	<0.01
hs-IL-6 (pg/ml)	4.70 (1.29-20.9)	3.72 (1.11-16.9)	<0.01
Creatinine (μmol/L)	83.8 (46.0-376)	76.5 (46.4-372)	0.83
eCrCl (ml/min)	73.5 (15.1-160)	79.7 (16.1-157)	0.15
Calorie intake (kJ)	1879.0 ± 953	1836.1 ± 1003	0.59
Total fat intake (g/day)	51.3 ± 33.4	50.4 ± 38.4	0.78
Tobacco users N (%)	127 (42.3)	137 (45.6)	0.41
Alcohol users N (%)	96 (32.0)	103 (34.3)	0.54

N indicates number of participants; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HR, heart rate; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; hs-CRP, high sensitivity C-reactive protein; hs-IL-6, high sensitivity interleukin 6, eCrCl, estimated creatinine clearance. Data are expressed as arithmetic mean ± standard deviation, geometric mean (5-95 percentiles) or % of N. All p values were obtained with independent T-tests, except for tobacco and alcohol users where Chi-square test was used.

The odds ratios of the HIV infected group versus the uninfected group is shown in Table 2. In this study population having a lower HDL-C is 5 times more likely when being a HIV

infected man and 3 times more likely in women. The odds ratio for having a higher TG, hs-CRP and hs-IL-6 level is respectively 1.7, 1.8 and 1.7 times more when being HIV infected.

Table 2

Odds Ratios of HIV infected (N = 300: M = 116, F = 184) participants vs. uninfected (N = 300: M = 116, F = 184) participants.

		OR	
		HIV infected vs. HIV uninfected	95% CI
WC	M (≥ 73 cm)	0.93	0.6-1.6
	F (≥ 75 cm)	0.94	0.6-1.4
TG	(≥ 1.0 mmol/L)	1.70	1.2-2.3*
HDL-C	M (< 1.4 mmol/L)	5.25	2.9-9.2*
	F (< 1.3 mmol/L)	2.92	1.9-4.5*
SBP	(≥ 124 mmHg)	0.72	0.5-1.0*
DBP	(≥ 84 mmHg)	0.72	0.5-1.0*
FG	(≥ 5.3 mmol/L)	0.64	0.5-0.9*
hs-CRP	(≥ 2.7 mg/L)	1.78	1.3-2.5*
hs-IL-6	(4.2 pg/ml)	1.67	1.2-2.3*

N indicates number of participants; M, male; F, female; WC, waist circumference; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FG, fasting glucose; hs-CRP, high sensitivity C-reactive protein; hs-IL-6, high sensitivity interleukin 6. Median of total group was used as cut-off value. Significance is indicated by *.

The MetS defined according to the ATP III and IDF criteria are shown for HIV infected and uninfected participants in Table 3. In comparison with the controls no difference (15.2% vs. 11.5%; $p = 0.18$) in the MetS was seen in the HIV infected, never ARV treated participants according to the ATP III definition. Similar results were shown for the IDF definition (21.1% vs. 22.6%; $p = 0.65$).

Table 3

Comparison of the prevalence of the MetS between the HIV infected (N = 300: M = 116, F = 184) and uninfected participants (N = 300: M = 116, F = 184).

		ATP III			IDF		
		HIV infected	HIV uninfected	<i>p</i>	HIV infected	HIV uninfected	<i>p</i>
WC	M	0.88	0.00	0.32	2.63	0.88	0.31
	F	18.33	18.68	0.93	33.9	40.1	0.22
TG		18.2	14.3	0.19	17.6	14.3	0.28
HDL-C	M	47.4	12.1	<0.01	46.5	11.2	<0.01
	F	62.6	33.7	<0.01	62.6	33.7	<0.01
BP		50.0	59.0	0.03	50.0	59.0	0.03
FG		22.7	25.1	0.49	36.6	43.7	0.08
MetS		15.2	11.5	0.18	21.1	22.6	0.65

N indicates number of participants; M, male; F, female; WC, waist circumference; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; BP, blood pressure; FG, fasting glucose; MetS, metabolic syndrome. Data are expressed as % of N. All *p* values were obtained via Chi-square test.

ATP III definition of MetS requires 3 or more of the following criteria: abdominal obesity, WC, men > 102 cm, women, > 88 cm; hypertriglyceridemia ≥ 1.69 mmol/L; low HDL-C, men < 1.04 mmol/L, women < 1.29 mmol/L; blood pressure $\geq 130/85$ mmHg, or fasting glucose ≥ 6.1 mmol/L.

IDF definition of MetS requires abdominal obesity, WC, men ≥ 94 cm, women ≥ 80 cm, and two or more of the following criteria: hypertriglyceridemia ≥ 1.7 mmol/L; low HDL-C, men < 1.03 mmol/L; women < 1.29 mmol/L; blood pressure $\geq 130/85$ mmHg, or fasting glucose ≥ 5.6 mmol/L.

DISCUSSION

In this study HIV-1 (subtype C) infected, never ARV treated, participants showed dyslipidemia and inflammation. These results are in agreement with documented data on HIV infected individuals of other population groups (12,18,29) where HIV-1 (subtype B) prevails (5).

A low HDL-C concentration increases the risk for coronary heart disease (30) and for every 1mg/dl increase in serum HDL-C a 2% reduction in CVD is estimated (31). Duprez *et al.* found that lower HDL levels were associated with a higher risk of CVD in HIV infected patients and Jericó *et al.* found smoking and HDL cholesterol to be the main cardiovascular risk factors in their HIV-infected study population (32,33). In our study population a HIV infected man is 7 times, and a woman 3 times more likely to have low HDL-C levels which should increase their risk for CVD. Even though Africans normally exhibit lower fasting triglyceride and higher HDL-C levels than Caucasians (34), HDL-C levels of our HIV infected participants (1.23 ± 0.58 mmol/L) were below the level of 1.28 mmol/L (50 mg/dl), which is seen as an increased risk for CVD (35).

Because the contribution of HIV to dyslipidemia is difficult to distinguish from those of classic cardiovascular risk factors, control participants were carefully matched by gender, age, BMI and locality to minimize the confounding effect of these non HIV related conditions on the results of this study. Furthermore, the participants in this study were newly identified and never received antiretroviral therapy. It could, therefore, be speculated that the metabolic changes seen have been influenced by HIV-1, subtype C, infection itself.

To identify the MetS in our study population the criteria of the most frequently used ATP III definition and more recent IDF definition were used. Although our infected participants never received antiretroviral treatment, the prevalence of MetS of 15% (ATP III definition) and 21% (IDF definition) is higher of that found by Badiou *et al.*, (36) in HIV positive individuals (7.3 vs. 11.2%; 80% treated by combined antiretroviral therapy) and in agreement with the study by Samaras *et al.* (14 vs. 18%), where 93% of the participants had received antiretroviral therapy at some time (37). The TG:HDL-C ratio, which is closely linked to lipid disorders associated with MetS, was higher in our HIV infected participants, as observed previously in HIV infected individuals (38) and in type 2 diabetes patients (39). The most prevalent lipid abnormalities seen in our study were the low HDL-C levels and higher TG levels exhibited by the HIV infected participants. This was also seen in the SIMONE study where the diagnosis of MetS in HIV infected individuals was mainly due to reduced HDL-C and high serum TG levels (40).

Much controversy still exists whether HIV infected individuals have a higher prevalence of MetS (with or without therapy). In the study of Mondy *et al.*, (41) the researchers found the prevalence of MetS to be similar among the HIV infected participants (25.5%), who received antiretroviral therapy, and the matched NHANES control participants (26.5%). On the other hand, in the cohort of HIV infected individuals of Samaras *et al.*, (37), the prevalence of the MetS was less in HIV positive adults (all received antiretroviral therapy at some time) than in the general population. Our study, where the participants never received antiretroviral therapy, shows that the prevalence of the MetS (both according to the ATP III and IDF definition) does not differ between the HIV infected and the matched uninfected participants. This finding is in contrast with the finding of Bonfanti *et al.*, which reported a higher prevalence of metabolic syndrome in never-treated HIV infected individuals than in the general population (23).

Abdominal obesity, an essential component of the MetS, seems to occur less frequently in HIV infected individuals (treated), and they seldom meet the cut-off value of the MetS criteria (37,42). Furthermore, there are no specific population group WC cut-off value available for

sub-Saharan Africans and in this study the cut-off value for Europeans were used, as suggested by the IDF definition, when identifying the prevalence of MetS using the IDF criteria. It is known that abdominal obesity is more prevalent among African women than men (43) and very few male participants met the WC cut-off value whether HIV infected (0.88% ATP III and 2.63% IDF) or not (0% ATP III and 0.88% IDF), suggesting that these WC cut-off values might influence the outcome of the prevalence of the MetS in our study. The fact that the participants of our study were matched according to BMI to differentiate between the contribution of the infection itself and classic risk factors could, therefore, not address this problem. Although more women met the WC cut-off value, it has also been shown that there is a clear difference regarding body composition between African and Caucasian women and Schutte *et al.* (44) suggested that these differences should be incorporated by the IDF definition when establishing a WC cut-off value for Africans. They also suggested that different HDL-C and TG cut-off values be used for Africans to determine the prevalence of MetS in an African population as they mostly show more favourable lipid values than Caucasians (44).

HIV lipodystrophy is characterized by dyslipidemia, visceral adiposity and a loss of abdominal and peripheral subcutaneous fat (45). Lipodystrophy is seen in only a small portion of HIV infected individuals not receiving antiretroviral therapy (46). The HIV infected and uninfected participants in this study were matched according to BMI and no differences were seen in waist or hip circumference or waist:hip ratio. Odds ratios also showed no increase in risk for an increase in waist circumference when being HIV infected. The participants were newly identified as being HIV infected and, therefore, it is not known how long the participants have been infected. However, it seems that no fat redistribution took place in our never-treated HIV infected participants.

The fasting glucose levels were similar between the HIV infected and the control groups. The use of fasting glucose level alone to diagnose impaired tolerance is likely to underestimate the prevalence of insulin resistance, advocated as a causative factor of the MetS (47).

Both low serum HDL-C levels and high serum hs-CRP levels are seen as independent risk factors for CVD (37,48), and the concentration of CRP is seen as a predictor of cardiovascular events (49). A study by Wadham *et al.* showed that HDL-C inhibits the inflammatory effect of CRP (50). In the general population the MetS is associated with inflammation and the IDF consensus group has highlighted elevated CRP as apparently being related to the MetS. The hs-CRP levels differ between the HIV infected and control

group (3.31 mg/L vs. 2.13 mg/L; $p = 0.0006$) and the OR for having a higher hs-CRP is 1.78 when HIV infected. The latter did not seem to influence the risk for MetS in this study. However, it is not known for how long the participants have been infected, and the duration of infection might be an important determinant of inflammatory status and its consequences.

Although hypertension is very common in black South Africans (51,52) and 59% of the HIV uninfected participants had a high blood pressure (according to both the ATP and IDF definition for MetS), the SBP as well as the prevalence of high blood pressure were lower in the HIV infected group. This seems to be consistent with previous studies (53), as hypertension in the HIV population is mostly associated with the use of ARV therapy, the metabolic syndrome, insulin resistance and/or anthropometric disorders (54).

Kidney function is often compromised in the HIV infected population, and HIV-associated nephropathy is frequently seen in immunosuppressed individuals of black ethnicity (55). In our study no difference in serum creatinine or estimated creatinine clearance rate was seen between the HIV infected and uninfected participants.

These results suggest that identification of the MetS in HIV-1 subtype C infected Africans should not be the main focus for the identification of individuals at risk for CVD. Low HDL-C and elevated TG levels seem to be the most prevalent abnormalities and therefore emphasis should be placed on determining the HDL-C and TG levels and to treat the metabolic abnormalities present, thereby reducing the probable cardiovascular risk faced by these individuals.

This study should be interpreted within the context of its strengths and limitations. A case-control design was applied and carefully matched the control subjects according to age, gender, BMI and locality. When viewing previous studies regarding HIV and cardiovascular risk, our study population is unique in specifically two aspects: they were infected with the subtype C virus instead of subtype B, and they were unaware of their infected status and, therefore, have never received ARV treatment. Thus the differences found could probably be attributed to the infection itself. Furthermore, the data as obtained in this study are limited in HIV infected South Africans. A limitation of the study is that the duration of the HIV infection is unknown as the participants were newly identified being HIV infected. Also, some of the participants chose not to visit the local clinic or hospital for follow-up (CD4 cell count determination and possible subsequent treatment) after they were informed of their HIV infected status. This is probably due to stigmatization which still exists among South African individuals (56,57).

In conclusion, the results of this study provide evidence that HIV-1, specifically subtype C, is associated with dyslipidemia and an inflammatory state of newly identified HIV infected, never-treated, African individuals that may increase their risk for cardiovascular disease. The study, therefore, shows that HIV-1 subtype C, though genetically different from subtype B, seems to influence the MetS components in the same way as HIV-1 subtype B, but does not increase the prevalence of the MetS in Africans.

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CHAPTER 3

**IS HIV-1 ASSOCIATED WITH ENDOTHELIAL DYSFUNCTION IN A
POPULATION OF AFRICAN ANCESTRY IN SOUTH AFRICA?**

IS HIV-1 ASSOCIATED WITH ENDOTHELIAL DYSFUNCTION IN A POPULATION OF AFRICAN ANCESTRY IN SOUTH AFRICA?

MOTIVATION FOR THE MANUSCRIPT

Cardiovascular involvement in various forms, such as dyslipidemia,¹⁻² lipodystrophy,³⁻⁴ endothelial dysfunction,⁵⁻⁶ accelerated atherosclerosis⁷ and coagulation disorders⁸ have been documented worldwide among HIV infected people. Endothelial dysfunction is considered a key factor in atherosclerosis-related diseases and is present in all stages of atherosclerosis.⁶ Furthermore, endothelial injury and dysfunction have been proposed as plausible links between HIV infection and atherosclerosis.⁹ The development of atherosclerosis may be the consequence of infection-triggered endothelial damage¹⁰ in the HIV infected population. Accelerated atherosclerosis has also been detected in HIV infected patients¹¹⁻¹² and a wide range of coagulation disorders may be associated with HIV infection itself.¹³ Endothelial dysfunction is characterized by an increased production of inflammatory cytokines and adhesion molecules¹⁴ and is also associated with low HDL levels among HIV infected people.⁶

In South Africa atherosclerotic disease, historically not common in most black Africans, is increasing.¹⁵ One of the important contributors, at least in part, to this increase could be the cardiac complications related to HIV infection. It has been recommended that HIV infection *per se* should count as a coronary risk factor, similar to traditional cardiovascular risk factors (smoking, hypertension, hypercholesterolemia, and diabetes)⁷ as this infection promotes chronic arterial inflammation that, in turn, promotes atherosclerosis and thrombosis. The role of HIV infection as a risk factor for premature atherosclerosis is still controversial.^{9,16} There is also uncertainty about the relative contribution of the viral infection, the virus itself, the associated inflammatory response, the antiretroviral therapy, and the interaction between them to the cardiovascular risk factors seen in the HIV infected population.^{5,17}

AIM OF THE MANUSCRIPT

The aim of this study was to assess whether newly identified, never-treated, HIV-1 infected South Africans of African ancestry show signs of inflammatory injury of the endothelium. This could lead to endothelial dysfunction, accelerated atherosclerosis and increased coagulation which could result in thrombosis.

HYPOTHESES OF THE MANUSCRIPT

- Inflammatory injury to the endothelium in the HIV-1 infected participants is indicated by increased the inflammatory markers CRP and IL-6.
- Endothelial dysfunction as indicated by an increase in atherosclerotic biological markers (ICAM and VCAM), and an increase in pro-thrombotic markers (fibrinogen and PAI) occur in the never-treated HIV-1 infected participants.

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IS HIV-1 ASSOCIATED WITH ENDOTHELIAL DYSFUNCTION IN A POPULATION OF AFRICAN ANCESTRY IN SOUTH AFRICA?

Running title: HIV-1 and endothelial dysfunction in South Africa

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ABSTRACT

The chronic infection status suffered by HIV infected individuals promotes chronic arterial inflammation and injury which promote dysfunction of the endothelium, atherosclerosis and thrombosis. HIV-1 subtype C is prevalent in South Africa and accounts for almost a third of the infections worldwide. This subtype differs genetically from HIV-1 subtype B on which the majority of studies have been done. The objective of this study was to assess whether newly identified, never-treated, HIV-1 infected South African participants show signs of endothelial dysfunction, accelerated atherosclerosis and increased coagulation. Three hundred newly diagnosed (never antiretroviral treated) HIV infected participants were compared to three hundred age, gender, body mass index and locality matched uninfected controls. High density lipoprotein cholesterol (HDL-C), triglycerides, interleukin-6 (IL-6), C-reactive protein (CRP), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and carotid radial pulse wave velocity (cr-PWV) were determined. The HIV infected participants showed lower HDL-C and higher IL-6, CRP, ICAM-1 and VCAM-1 levels compared to the uninfected controls ($p < 0.05$). No difference in fibrinogen and PAI-1 was detected. A continuous positive trend of increasing age with cr-PWV was detected only in the HIV infected group. Our findings suggest inflammatory injury of the endothelium, pointing to

endothelial dysfunction of never-treated HIV-1 infected South Africans of African ancestry. Although no indication of a prothrombotic state could be detected, there is an indication of accelerated vascular aging and probable early atherosclerosis in the older HIV infected participants.

Keywords: HIV-1, South Africa, endothelial dysfunction, vascular aging, never-treated, inflammation.

INTRODUCTION

Several cardiovascular risk factors have been associated with, or observed in the human immunodeficiency virus (HIV) infected individuals due to a longer life expectancy after antiretroviral (ARV) treatment.¹⁻² Worldwide cardiovascular involvement in various forms, such as endothelial dysfunction,³ accelerated atherosclerosis⁴ and coagulation disorders⁵ have been documented among HIV infected individuals. In South Africa atherosclerotic disease, historically not common in black Africans, is increasing.⁶ One of the important contributors to this increase could be the cardiac complications related to HIV infection, as South Africa is the country with the highest HIV infections in the world.⁷

The chronic infection of HIV infected individuals promotes chronic arterial inflammation and injury, that in turn promotes dysfunction of the endothelium, atherosclerosis and thrombosis.^{5,8} Endothelial injury and dysfunction have been proposed as plausible links between HIV infection and atherosclerosis.⁹ The development of atherosclerosis may be the consequence of infection-triggered endothelial damage,¹⁰ and atherosclerotic cardiovascular events are commonly manifested via thrombotic events.¹¹ Increased levels of the inflammatory markers C-reactive protein (CRP), interleukin 6 (IL-6)⁸ and cell adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1),³ have been reported in the HIV infected population.^{2,12} Accelerated atherosclerosis has also been detected in HIV infected patients^{1,13} and a wide range of coagulatory disorders may be associated with HIV infection itself.⁵

Although an estimated 5.5 million people are living with HIV in South Africa⁷ where HIV-1 subtype C prevails,¹⁴⁻¹⁵ the majority of studies on HIV have been done on HIV-1 subtype B responsible for infections in North America, Europe and Australia.¹⁵⁻¹⁶ Subtype C accounts for 55-60% of all HIV infections worldwide and differs as much as 30% in its genome from subtype B.^{17,18} The clinical consequences of subtype variations is still unknown and the effect of the HIV-1 subtype C virus on the vascular system is not clear.

Recently it was recommended that HIV infection *per se* should count as a coronary risk factor, similar to traditional cardiovascular risk factors (smoking, hypertension, hypercholesterolemia, and diabetes).⁴ Although data of Lorenz *et al.* support the hypothesis that HIV infection promotes early atherosclerosis independently of the "classic" vascular risk factors,¹³ the role of HIV infection as a risk factor for premature atherosclerosis is still controversial.^{9,19} There is also uncertainty about the relative contribution of the viral infection, the virus itself, the associated inflammatory response, the antiretroviral therapy,

and the interaction between them to the cardiovascular risk factors seen in the HIV infected population.^{20,21}

In view of the above the aim of this study was to assess whether newly identified, never-treated, HIV-1 infected South Africans of African ancestry show signs of endothelial dysfunction, accelerated atherosclerosis and increased coagulation which could lead to thrombosis.

MATERIALS AND METHODS

Study design and participants

This sub-study is nested in the larger international PURE (Prospective Urban and Rural Epidemiology) study. The international PURE study is a longitudinal multinational study that will address questions regarding the cause and development of cardiovascular risk factors and chronic disease within populations in developing countries, including South Africa²². The South African leg of the study was performed in the North West Province where a total of 2000 participants (1000 urban and 1000 rural) were randomly recruited from a rural and urban setting and screened during the baseline phase in 2005. The inclusion criteria were volunteers older than 35 years that were non-users of any chronic medication and with no self-reported diseases. For this case-control sub-study 300 newly identified HIV infected participants of the baseline PURE study population were individually matched with 300 HIV uninfected participants, according to age, gender, body mass index (BMI) and locality (urban and rural). The protocol appropriate to this sub-study will be discussed.

Ethical considerations

All participants provided signed informed consent after all procedures were explained to them in their home language. The study protocol complies with the Declaration of Helsinki as revised in 2004²³ and was approved by the Ethics Committee of the North-West University, Potchefstroom, South Africa.

Experimental protocol

Permission to execute the study was obtained from the provincial Department of Health, local authorities and from the Tribal Chief in the rural area. Over a period of twelve weeks 30-35 participants arrived at the research locality of the rural or urban areas daily at about 07:00 each morning after a 10-15 minute drive (provided by the research team) from their communities. The participants were introduced to the setup and after the procedures were explained they signed the informed consent forms and received HIV pre-counseling given by

trained counselors. The HIV status of the participants was revealed during individual post-counseling and the infected participants were referred to their local clinics or hospitals for follow-up and CD4 cell count determination. During the course of the morning, demographic, lifestyle and food frequency questionnaires were completed with the help of the specially trained field workers in the subjects' home language. Lifestyle data included self-reported current tobacco use, alcohol intake as well as medical history.

Anthropometric measurements

Height, weight, hip and waist circumference (WC) were measured (Precision Health Scale, A & D Company, Japan; Invicta Stadiometer, IP 1465, UK; Holtain unstretchable metal tape) using standardized procedures.²⁴

Cardiovascular measurements

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained with the validated OMRON HEM-757 device. After a 10 minute rest period blood pressure measurements were performed twice (5 minutes apart) on the right arm (brachial artery), while the participants were seated upright and relaxed with his/her right arm supported at heart level. Appropriate cuffs were used for obese participants. Mean arterial pressure (MAP) pressure was calculated by diastolic blood pressure + $\frac{1}{3}$ pulse pressure.

Carotid-radialis pulse wave velocity (cr-PWV) was measured on the left side of each participant in the supine position by making use of the Complior SP apparatus (Artech-Medical, Pantin, France).

Blood, serum and plasma samples

Fasting blood samples were obtained from the antebrachial vein using a sterile winged infusion set and syringes. Serum and plasma were prepared according to appropriate methods and stored at -80°C in the laboratory. In the rural area serum/plasma was stored at -18°C (no longer than five days) until it could be transported to the laboratory facility and was then stored at -80°C until analysis.

Biochemical analyses

Quantitative determination of high density lipoprotein cholesterol (HDL-C), triglycerides (TG), high sensitivity C-reactive protein (hsCRP), glucose and creatinine concentration in the serum of the participants were done with the Konelab™ auto analyzer (Thermo Scientific, Vantaa, Finland), which is a clinical chemistry analyzer for colorimetric, immunoturbidimetric and ion-selective electrode measurements. Creatinine clearance rate was estimated using the Cockcroft-Gault formula.

Serum concentrations of high sensitivity interleukin-6 (hsIL-6) were measured using human enzyme-linked immunosorbent assays (Quantikine® HS ELISA, R&D Systems, Minneapolis, USA). Concentrations of serum intercellular adhesion molecule 1 (sICAM-1) and serum vascular cell adhesion molecule 1 (sVCAM-1) were assessed by sandwich ELISAs (human sICAM-1 and human sVCAM-1 assay, IBL, Hamburg, Germany).

The quantitative determination of fibrinogen in plasma was performed by the Multifibren U-test (Dade Behring), a modification of the Clauss method on the Dade Behring BCS coagulation analyzer. The quantification of plasminogen activator inhibitor-1 (PAI-1) activity was performed by a chromogenic assay kit, Spectrolyse®/pL PAI-1 (Trinity Biotech plc, Bray Co, Ireland).

HIV status was determined according to the protocol of the National Department of Health of South Africa. A rapid card test, First Response (PMC Medical, India) was used for testing, using whole blood. If tested positive, the result was repeated with the Pareeshak (BHAT Bio-tech India) card test for confirmation. The HIV-1 subtype C epidemic prevalent in South Africa has been established by serotyping and genotyping.^{7,14-15} The CD4 cell counts were obtained from the local clinic or hospital within three months from the data collection of the baseline phase. CD4 cell counts could only be obtained for 72 participants as these were the only participants who visited their local clinic or hospital for follow-up. The CD4 counts were determined (in whole blood) by the National Health Laboratory using flow cytometric analysis (Beckman COULTER® EPICS® XL™, Fullerton, USA).

Statistical analysis

All data were statistically analyzed by means of Statistica v.8 (Statsoft Inc., OK, USA, 2008). Mean values, standard deviations and standard errors were calculated. The distribution of hsCRP, hsIL-6, sICAM-1, sVCAM-1, fibrinogen and PAI-1 were normalized by logarithmic transformation before analysis, reporting the geometric mean and the 5th and 95th percentile intervals. Independent T-tests were used to compare the uninfected group with either the infected group or the group with the nadir CD4 cell count. ANOVA and Tukey's posthoc test for multiple comparisons were used to compare the characteristics of the continuous variables of the HIV uninfected, infected and nadir CD4 cell count groups. The Chi-square tests were done to compare data of categorical variables. An analysis of covariance (ANCOVA) and Bonferroni posthoc test were performed to compare the inflammatory and cell adhesion biomarkers of the infected and uninfected groups, whilst adjusting for MAP, tobacco and alcohol use. Partial correlations were performed in the HIV uninfected, infected and nadir CD4 cell count groups, whilst adjusting for MAP, tobacco and alcohol use. For the cr-PWV analysis the subjects were divided into three age groups (with 10 year intervals;

group 1: <40 years, group 2: 40-50 years and group 3: >50 years) and ANCOVA (adjusted for gender, BMI, MAP, tobacco and alcohol use) was performed. The cr-PWV was plotted for the age groups for the HIV infected and uninfected participants.

RESULTS

The characteristics of the HIV infected participants and matching controls as well as the subgroup of the HIV infected participants with a nadir CD4 cell count < 200 cells/mm³ are reported in Table 1. Due to individual matching, age and BMI values were identical in the HIV infected and uninfected (control) groups. The SBP and HDL-C were lower and the TG, TG:HDL-C ratio, hsIL-6, hsCRP, sICAM-1 and sVCAM-1 were higher in the HIV infected compared to the uninfected participants. The lowest HDL-C levels were seen in the HIV infected participants with a nadir CD4 cell count. The mean levels of hsIL-6, hsCRP, sICAM-1 and sVCAM-1 were the highest in the nadir CD4 cell count group and, apart from hsIL-6, differed significantly when the nadir group was compared to the uninfected group. When the low HDL-C and high hsIL-6, hsCRP, sICAM-1 and sVCAM-1 of the nadir CD4 cell count group were compared to the infected participants with a CD4 cell count > 200 cells/mm³ only the sVCAM-1 differed significantly (p=0.046, results not shown). After adjustments for MAP, alcohol and tobacco the overall results did not change. No gender differences were seen.

Table 1

Characteristics of participants: HIV uninfected, HIV infected and HIV infected participants with a nadir CD4 cell count < 200 cells/mm³.

	HIV uninfected N = 300	<i>p</i> -Value [†] HIV uninfected / infected	HIV infected N = 300	<i>p</i> -Value [†] HIV uninfected / nadir CD4 cell count	Nadir CD4 cell count N = 18	<i>p</i> -Value [‡] trend
Age (years)	44.0 ± 7.81	0.971	44.0 ± 8.04	0.679	44.8 ± 8.48	0.904
Men / women N	116/184	1.000	116/184	0.135	10/8	0.316
Body mass index (kg/m ²)	22.8 ± 5.48	0.916	22.9 ± 5.59	0.122	20.8 ± 3.96	0.258
Systolic blood pressure (mm Hg)	129 ± 21.8	0.003	124 ± 21.8	0.309	124 ± 17.5	0.015
Diastolic blood pressure (mm Hg)	85.9 ± 14.3	0.099	84.0 ± 14.7	0.145	80.9 ± 10.6	0.191
Mean arterial pressure (mm Hg)	100 ± 16.1	0.024	97.5 ± 16.7	0.179	95.3 ± 12.1	0.179
Carotid radial pulse wave velocity (m/s)	10.9 ± 2.30	0.606	11.1 ± 2.10	0.307	11.5 ± 2.30	0.561
Lipids: HDL-C (mmol/L)	1.70 ± 0.71	<0.001	1.23 ± 0.58	<0.001	1.07 ± 0.47	<0.001
TG (mmol/L)	1.15 ± 0.75	0.031	1.29 ± 0.77	0.814	1.19 ± 0.76	0.085
TG:HDL-C ratio	0.86 ± 1.21	<0.001	1.41 ± 1.47	0.006	1.19 ± 0.21	<0.001
Inflammatory markers: hsIL-6 (pg/ml)	3.72 (1.11-16.9)	<0.001	4.70 (1.29-20.9)	0.124	5.03 (1.10-22.3)	0.002
hsCRP (mg/L)	2.13 (0.23-29.2)	<0.001	3.31 (0.32-50.4)	0.012	5.34 (0.56-62.1)	0.001
sICAM-1 (ng/ml)	405 (111-1345)	<0.001	577 (192-1610)	0.009	696 (194-1884)	<0.001
sVCAM-1 (ng/ml)	397 (19-2252)	<0.001	847 (101-3230)	0.001	1262 (143-3421)	<0.001
Coagulation markers: Fibrinogen (g/L)	2.99 (1.39-7.19)	0.041	2.75 (1.29-6.99)	0.785	3.11 (1.39-9.00)	0.073
PAI-1 (IU/ml)	1.39 (0.01-17.3)	0.681	1.52 (0.01-18.8)	0.433	0.83 (0.01-17.4)	0.563
Glucose (mmol/L)	5.50 ± 1.10	0.126	5.35 ± 1.26	0.052	4.97 ± 0.29	0.122
eCrCl (ml/min)	79.7 (16.1-157)	0.150	73.5 (15.1-160)	0.790	76.5 (9.58-163)	0.345
Tobacco users N (%)	137 (45.6)	0.410	127 (42.3)	0.082	12 (66.7)	0.071
Alcohol users N (%)	103 (34.3)	0.543	96 (32.0)	0.693	7 (38.9)	0.678

N indicates number of participants; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; hsIL-6, high sensitivity interleukin 6; hsCRP, high sensitivity C-reactive protein; sICAM-1, serum intercellular adhesion molecule-1; sVCAM-1, serum vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1. Data are expressed as arithmetic mean $\pm$ standard deviation, geometric mean (5th and 95th percentile intervals) or % of N. *p*-Values[†] between uninfected/infected and uninfected/nadir CD4 cell count were obtained with independent T-test. *p*-Values[‡] trend were obtained with ANOVA, and for gender, tobacco and alcohol users Chi-square test was used.

The odds ratios of the HIV infected group versus the uninfected group are shown in Table 2. In this study population having a lower HDL-C is 3.7 times more likely in the HIV infected participants. The odds ratio for having higher hsCRP, TG, TG:HDL ratio, hs-IL-6, sICAM-1 and sVCAM-1 levels is respectively 1.8, 1.7, 3.3, 1.7, 2.0 and 3.9 times more in the HIV infected participants.

Table 2

Odds ratios of HIV infected participants vs. uninfected participants.

		Odds ratios	
		HIV infected versus HIV uninfected	95% CI
HDL-C	< 1.36 mmol/L	3.69	2.6-5.2*
TG	≥ 1.0 mmol/L	1.70	1.2-2.3*
TG:HDL ratio	≥ 0.75	3.33	2.4-4.7*
hsCRP	≥ 2.7 mg/L	1.78	1.3-2.5*
hsIL-6	≥ 4.2 pg/ml	1.67	1.2-2.3*
sICAM-1	≥ 516 ng/ml	2.04	1.5-2.8*
sVCAM-1	≥ 693 ng/ml	3.92	2.8-5.5*

HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; TG:HDL, triglycerides - high-density lipoprotein ratio; hsCRP, high sensitivity C-reactive protein; hsIL-6, high sensitivity interleukin 6; sICAM-1, serum intercellular adhesion molecule-1; sVCAM-1, serum vascular cell adhesion molecule-1. For all variables the median of total group was used as cut-off value. Significance is indicated by *.

Partial correlations (adjusted for MAP, tobacco and alcohol use) with a p value < 0.05 are listed in Table 3. HDL-C correlated inversely with TG in all 3 groups ($r=-0.23$, $p<0.001$; $r=-0.16$, $p=0.005$; $r=-0.55$, $p=0.41$), with log sICAM-1 in the uninfected ($r=-0.14$, $p=0.02$) and infected group ($r=-0.15$, $p=0.009$), and with hsIL-6 only in the HIV infected group ($r=-0.21$, $p=0.001$). In the nadir CD4 cell count group, the CD4 cell count was inversely correlated with hsCRP ($r=-0.63$, $p=0.01$) and fibrinogen ($r=-0.78$, $p=0.001$).

Table 3

Partial correlation coefficients between the different variables of the HIV uninfected, infected and nadir (<200 cells/mm³) CD4 cell count groups.

Variables	HIV uninfected (N =300)							HIV infected (N =300)						Nadir CD4 cell count (N =18)			
	Age	cr-PWV	HDL-C	TG	Log hslL-6	Log hsCRP	Log sICAM-1	Age	HDL-C	TG	Log hslL-6	Log hsCRP	Log sICAM-1	CD4	Age	HDL-C	Log hsCRP
Age																	
cr-PWV								0.14									
HDL-C																	
TG			-0.23					0.14	-0.16							-0.55	
Log hslL-6									-0.21								
Log hsCRP						0.45					0.52			-0.63			
Log sICAM-1			-0.14				0.14		-0.15	0.12	0.17	0.14					
Log sVCAM-1							0.27						0.30				
Fibrinogen		-0.12		-0.17	0.29	0.45					0.24	0.33		-0.78			0.58
Log PAI-1				0.17						0.18					-0.67		
Log eCrCl	-0.14							-0.25									

N indicates number of participants; cr-PWV, carotid radialis pulse wave velocity; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; hslL-6, high sensitivity interleukin 6; hsCRP, high sensitivity C-reactive protein; sICAM-1, serum intercellular adhesion molecule-1; sVCAM-1, serum vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; eCrCl, estimated creatinine clearance. Adjustments were made for mean arterial pressure, tobacco and alcohol use. Only significant ($p < 0.05$) correlation coefficients given.

Age correlated positively with cr-PWV only in the HIV infected group ($r=0.14$, $p=0.01$) after adjusting for MAP as well as tobacco and alcohol use. When the participants were divided into age groups with 10 year intervals, and after adjusting for gender, BMI, MAP, tobacco and alcohol use, a continuous positive trend of increasing cr-PWV with age ($p=0.09$) was detected only in the HIV infected group (Figure 1). In the age group older than 50 years, the cr-PWV between the infected and uninfected groups differed ($p=0.057$).

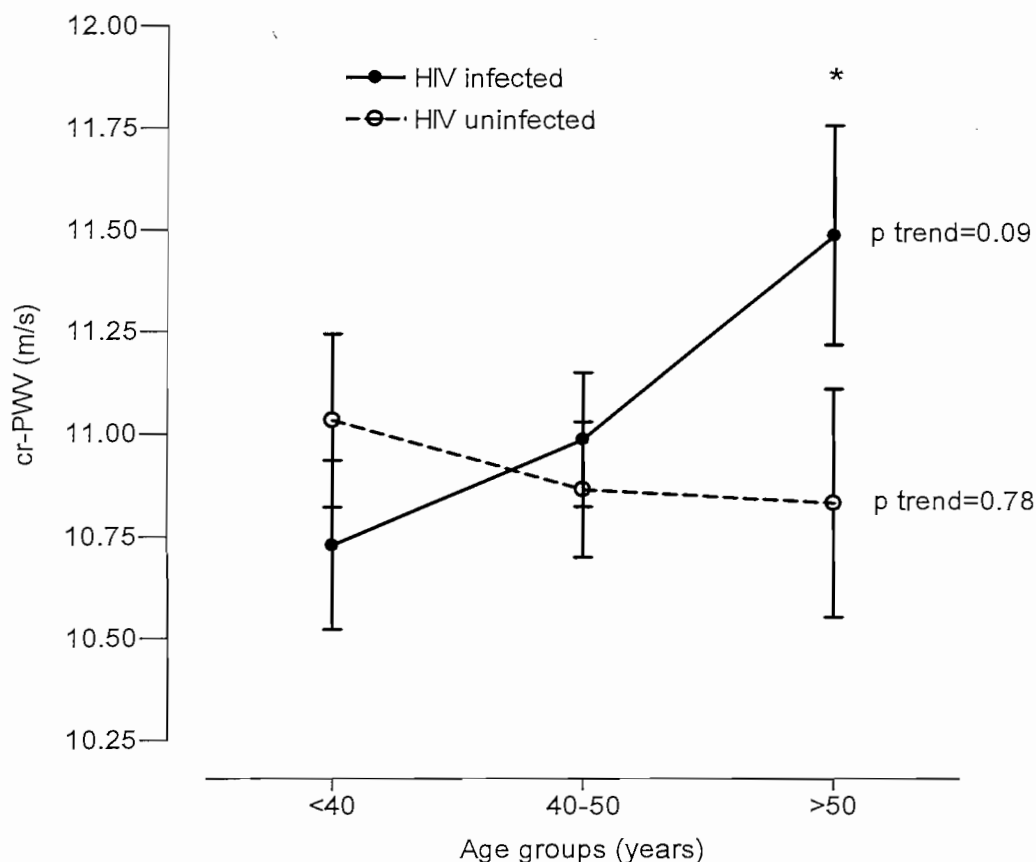


Fig. 1. cr-PWV in the HIV infected group and uninfected group with increasing age. Adjusted for gender, BMI, MAP, self-reported alcohol and tobacco use. Values are means $\pm$ SEM.
* : cr-PWV of HIV infected and uninfected participants differ ($p=0.057$).

DISCUSSION

In this case-control study the HIV infected participants, who have never received antiretroviral therapy, showed lower HDL-C and higher hsIL-6, hsCRP, sICAM-1 and sVCAM-1 levels than their age, gender, BMI and locality (urban, rural) matched controls. The higher levels of inflammatory markers and low HDL-C levels could point to endothelial dysfunction which is seen as the link between infection, inflammation and atherosclerosis.¹⁰ Furthermore, in the older HIV infected participants a positive trend of increasing peripheral

arterial stiffness was detected, which was not observed in the uninfected participants. This could indicate accelerated vascular aging in these participants. No difference in coagulation factors was detected between the infected and uninfected groups.

The contribution of HIV to endothelial dysfunction is difficult to distinguish from traditional cardiovascular risk factors, therefore, the control participants' gender, age, BMI and locality carefully matched to minimize the confounding effect of these conditions on the study findings.

In epidemiological studies high plasma levels of HDL-C protect against the development of atherosclerosis.²⁵ Besides HDL-C's known ability to promote the efflux of cholesterol from cells in the arterial wall,²⁶ and thereby maintaining cell cholesterol homeostasis, HDL-C is also antithrombotic,²⁷ and possesses antioxidant and anti-inflammatory properties.²⁸⁻²⁹ Our HIV infected participants had lower serum HDL-C levels and were 3.7 times more likely to have low HDL-C levels than their matched controls, while the lowest levels were seen in the nadir CD4 cell count group. The protective effect of HDL-C is lost in our HIV infected group due to the low levels. Furthermore, Patel *et al.* demonstrated that triglycerides contribute to atherosclerosis-mediated inflammation, by direct effect on the endothelium and also potentially by attenuating the protective effects of HDL-C against vascular inflammation.³⁰ It could, therefore, be expected that the low serum HDL-C and high TG levels – thus a higher TG/HDL-C ratio - seen in the HIV infected versus uninfected participants, worsen the inflammation as reflected by the higher levels of, and odds ratios for having higher levels of hsIL-6 and hsCRP in the infected group. The inverse correlation of HDL-C with hsIL-6 ($r=-0.21$, $p=0.001$), only seen in our HIV infected group, further emphasizes the inflammation in this group. Clinical and epidemiological studies showed that HDL-C concentration is often inversely related with plasma levels of cytokines in atherosclerotic cardiovascular diseases.³¹

While IL-6 is an early stimulator of the inflammatory process and CRP is produced in response to IL-6 secretion, CRP is thought to induce ICAM and VCAM secretion.³² These adhesion molecules are known to be expressed in arteries *in vivo* at sites of developing atherosclerosis,³³ and indicate vascular endothelium injury and endothelial dysfunction.^{3,20,34} The CRP-induced expression of endothelial adhesion molecules is inhibited by HDL-C.³¹ The HIV infected participants in our study showed lower HDL-C and higher hsIL-6, hsCRP, sICAM-1 and sVCAM-1 levels compared to their uninfected controls. The inflammatory process is thereby clearly activated, resulting in endothelial injury. The HIV infected participants were 2 and 4 times more likely to express higher levels of sICAM-1 and sVCAM-1 respectively, further indicating endothelial injury in this group. It was found that HIV-1 Tat

protein (thus HIV itself) induced the expression of ICAM-1 and VCAM-1 and this could be a possible mechanism by which HIV-1 infection contributes to endothelial injury and accelerated atherosclerosis.³⁵⁻³⁶ It could, therefore, be expected that the HIV infected, never antiretroviral treated participants, with definite signs of endothelial injury would also show signs of endothelial dysfunction.

Endothelial dysfunction results in increased arterial stiffness³⁷ which increases as arteries become more damaged.³⁸ Using femoral PWV previous studies showed increased aortic (central) stiffness in treated³⁹ and untreated⁹ HIV infected individuals. In our study the peripheral cr-PWV did not differ between the HIV infected participants compared to their age, gender, BMI and locality matched controls. However, age correlated positively (although weak) with carotid radial PWV ($r=0.14$, $p=0.01$) in the HIV infected group alone. Age is one of the principal factors modulating PWV⁴⁰ which is the greatest in the elastic aorta, and least in muscular arteries such as those of the upper limb.⁴¹ When divided into 10 year interval age groups, our HIV infected participants showed a positive trend of increasing peripheral cr-PWV with age. Since changes in PWV in muscular arteries are not normally found with increasing age,⁴² (as in the uninfected group of the present study), this increase in PWV in muscular arteries of the HIV infected participants is an interesting result. Although increased stiffness could indicate atherosclerosis, *per se*, over and above that due to aging,⁴³ our results being in muscular arteries may point to premature vascular aging in the HIV infected participants. This is in agreement with the results of Lorenz *et al.* who obtained a higher "vascular" age of 4-5 years for HIV infected patients (treated) compared to controls.¹³ These results indicate that HIV-1, without the effect of treatment, might contribute to accelerated vascular aging and possible early atherosclerosis.

Endothelial dysfunction is also associated with a prothrombotic state,⁴⁴ and studies which investigated the prothrombotic state in HIV infected populations showed increased levels of PAI-1 activity and fibrinogen.^{4,45-46} However, in our study neither the PAI-1 activity nor fibrinogen was increased in the HIV infected subjects, indicating no signs of a prothrombotic state. Our findings are in agreement with the findings of James *et al.*, who found that HIV infection was not associated with the fibrinogen concentration in Africans.⁴⁷ It is known that black South Africans of African ancestry have high levels of fibrinogen⁴⁸ and it may, therefore, be that ethnic effects on plasma fibrinogen may have masked the potential effect of HIV.

This study has limitations and strengths. The study has a case-control design and control participants were carefully matched to the infected participants according to age, gender,

BMI and locality. When viewing previous studies regarding HIV and cardiovascular risk, this study population is unique as they were unaware of their infected status and, therefore, have never received antiretroviral treatment. Thus, although the evidence of no self-reported diseases was not evaluated, the differences found could probably be attributed to the infection itself and not the ARV treatment. Furthermore, although South Africa is the country with the highest infection rate in the world,⁷ data on cardiovascular changes and risk in HIV-1 infected South Africans are very limited.

A limitation of the study is that the participants did not visit the local clinic or hospital for follow-up and CD4 cell count determination. This was probably due to stigmatization which still exists among South African individuals,^{49, 50} and other aspects such as around the illness itself and poverty.⁵¹ Therefore, the sample size of the nadir CD4 cell count group is very small and those results should be interpreted with caution. Also, the subjects in this study were newly identified as being HIV infected and therefore the duration of the infection is not known. The lack of increase in fibrinogen, PAI-1 and cr-PWV in the infected group may be related to the most likely short duration of the infection as is also speculated in the study of James *et al.*⁴⁷ This is confirmed by the tendency of increased (though not statistically significant) fibrinogen in the nadir CD4 cell count group. A longitudinal study is therefore proposed to investigate further the influence of HIV on the endothelium and prothrombotic state of Africans. A recommendation for future studies would be to perform carotid intima media thickness measurements to verify endothelial damage and probable atherosclerosis.

To conclude, the findings suggest inflammatory injury of the endothelium, pointing to endothelial dysfunction, of never antiretroviral treated, HIV-1 infected South Africans. Attenuation of the protective effect of HDL-C probably worsened the endothelial inflammation. Although there was no indication of a prothrombotic state which could result in atherosclerotic disease, there is an indication of accelerated vascular aging and probable early atherosclerosis in the older HIV infected participants.

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DISCLOSURE STATEMENT

No competing financial interests exist.

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CHAPTER 4

**SuPAR IS ASSOCIATED WITH METABOLIC CHANGES IN HIV-1
INFECTED AFRICANS THREE YEARS AFTER ENROLLING FOR
TREATMENT**

SuPAR IS ASSOCIATED WITH METABOLIC CHANGES IN HIV-1 INFECTED AFRICANS THREE YEARS AFTER ENROLLING FOR TREATMENT

MOTIVATION FOR THE MANUSCRIPT

HIV infection, the associated chronic inflammation, and especially the use of antiretroviral therapy, are associated with an increased risk for cardiovascular disease,¹ an increase in insulin resistance, dyslipidemia,² lipodystrophy,^{3,4} endothelial dysfunction,⁵ accelerated atherosclerosis⁶ and coagulatory disorders.⁷ Researchers associated elevated blood suPAR levels with inflammation and progression of disease in several conditions including HIV-1.⁸ Elevated blood suPAR levels were also associated with lipodystrophy in HIV-1 infected patients on Highly Active Antiretroviral Therapy (HAART).⁹ Furthermore, suPAR was independently related to intima-media thickness (IMT) in uraemic patients,¹⁰ indicating a possible association between suPAR and the development of cardiovascular disease.

The antiretroviral roll-out programme, introduced in February 2004 in South Africa,¹¹ led to a decline in the HIV-1 related mortality.¹² However, the effect of the treatment on the cardiovascular system and metabolic status of the South African HIV infected population still needs to be determined.

AIMS OF THE MANUSCRIPT

- The first aim was to determine whether suPAR levels are elevated in HIV-1 infected black South Africans who enrolled for antiretroviral therapy and those who did not enroll, compared to uninfected controls before and after a three year follow-up.
- The second aim was to investigate whether suPAR correlated with similar cardiovascular and metabolic changes over a three year period in the HIV-1 infected individuals, treated and never-treated.

HYPOTHESES OF THE MANUSCRIPT

- The suPAR levels are increased in HIV-1, most likely subtype C, infected participants.
- The suPAR levels are decreased in treated HIV-1 infected participants.
- SuPAR correlates with similar cardiovascular and metabolic changes in both treated and never-treated HIV-1 infected participants after a three year period.

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SuPAR IS ASSOCIATED WITH METABOLIC CHANGES IN HIV-1 INFECTED AFRICANS THREE YEARS AFTER ENROLLING FOR TREATMENT

Running title: SuPAR among HIV-1 infected Africans

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ABSTRACT

Objectives: The novel biomarker, soluble urokinase Plasminogen Activator Receptor (suPAR), is associated with inflammation and progression of disease and may predict lipodystrophy and dysmetabolism in human immunodeficiency virus (HIV) infected individuals in the antiretroviral therapy era. We aimed to assess whether suPAR levels are elevated in treated and untreated HIV-1 infected Africans compared to uninfected controls before and three years after the introduction of antiretroviral therapy (ART) and whether baseline suPAR levels correlated with cardiovascular and/or metabolic changes.

Methods: In this prospective study 154 uninfected and 140 HIV-1 infected normo-glycemic African individuals (77 never-treated and 63 treated) were followed-up after 3 years. SuPAR, cardiovascular and metabolic variables were assessed and the percentage change over the three year period was determined.

Results: After 3 years the treated HIV-1 infected participants showed signs of lipodystrophy and a greater increase in suPAR levels compared to the never-treated and uninfected participants ($p=0.02$). In the treated HIV-1 infected group baseline suPAR levels correlated positively with an increased waist circumference and inversely with increased TC:HDL-C ratio. The never treated HIV-1 infected participants showed no increase in suPAR levels and dyslipidemia when compared to the uninfected control group.

Conclusions: After three years treated HIV-1 infected Africans had a larger increase in suPAR than never-treated or uninfected Africans. Baseline suPAR correlated with the development of lipodystrophy only in the treated group.

Keywords: suPAR, HIV-1, antiretroviral therapy, lipodystrophy, black South Africans

INTRODUCTION

The novel biomarker soluble urokinase Plasminogen Activator Receptor (suPAR) is a stable plasma protein¹ expressed predominantly by leukocytes,² and is associated with inflammation and progression of disease.³ It is not fully elucidated whether the poor overall outcome associated with elevated blood levels of suPAR in human immunodeficiency virus (HIV) infected patients is caused by a direct association between HIV and the components of the uPAR system, or simply mirror inflammation.⁴ However, blood levels of suPAR were linked to inflammation and immune activation.⁴

Antiretroviral therapy (ART) which was introduced in the late 1990's⁵ in Europe and North America where HIV-1 subtype B prevails^{6,7} reduced the morbidity and mortality associated with HIV. However, HIV infection, and especially the use of antiretroviral therapy, is associated with an increased risk for cardiovascular disease (CVD),⁸ an increase in insulin resistance, dyslipidemia,⁹ lipodystrophy,^{10,11} endothelial dysfunction,¹² accelerated atherosclerosis¹³ and coagulation disorders.¹⁴

Lipodystrophy was associated with a 70% increase in plasma suPAR levels in treated HIV infected patients on highly active antiretroviral therapy (HAART).¹⁵ Thus it was suggested that suPAR may be a marker linking immunologic, inflammatory, and metabolic characteristics (lipid and glucose metabolism, as well as fat redistribution) of HIV infected patients on HAART.¹ SuPAR levels were elevated in patients with cardiovascular disease undergoing hemodialysis,¹⁶ and independently related to intima-media thickness (IMT) in uremic patients indicating an involvement in atherosclerosis.¹⁷

In South Africa HIV-1, most likely subtype C, which accounts for 55-60% of all HIV infections worldwide, prevails.¹⁸ The antiretroviral roll-out programme was introduced in February 2004 giving HIV infected individuals access for the first time to free antiretroviral therapy.¹⁹ This led to a decline in the HIV related mortality,²⁰ but the effect of treatment on the cardiovascular and metabolic status of the South Africa HIV-1 infected population still needs to be determined.

We aimed to determine whether suPAR levels are elevated in HIV-1 infected black South Africans who did enroll for antiretroviral therapy and those who did not enroll, compared to uninfected controls before and after a three-year follow-up. The second aim was to investigate whether baseline suPAR levels correlated with similar cardiovascular and metabolic changes over the three year period in the HIV-1 infected individuals, treated and never-treated.

METHODS

Study design and participants

This sub-study is embedded in the international PURE (Prospective Urban and Rural Epidemiology) study. The PURE study is an epidemiological study that will address questions regarding the cause and development of cardiovascular risk factors and disease within populations with comparable coverage, particularly of low and middle income countries, including South Africa.²¹ A minimum follow-up of 10 years is currently planned. The baseline data collection of the South African leg of the study was performed in the North West Province in 2005. For this sub-study a prospective study was performed aimed at evaluating the changes in metabolic and cardiovascular profiles before and after introduction of antiretroviral therapy of participants newly identified as being HIV infected in 2005 and their uninfected controls. The methodology appropriate to the sub-study will be discussed.

The outline of the study is shown in Figure 1. During the PURE South Africa baseline study (2005) the participants were unaware of their HIV status and 300 participants were newly identified as being HIV infected and had never received antiretroviral therapy. After being identified as HIV infected, the participants were referred to their nearest hospital or clinic for follow-up on the HIV infection diagnosis. In South Africa antiretroviral therapy is started at a CD4 cell count of ≤ 200 cells/mm³, and as many developing countries, South Africa uses the World Health Organization's recommendation of two nucleoside reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI) as first-line therapy.^{19,22} The first line therapy of the participants in our study comprised of stavudine and lamivudine plus efavirenz or nevirapine and was supplied free of charge as part of the roll-out programme. Thus the group receiving antiretroviral therapy (ART group) in November 2008 (N=63, Figure 1) was newly identified as being HIV infected during the baseline study in November 2005. Twenty of these participants received the first line therapy during 2006 as their CD4 cell count was below 200 cells/mm³, while the other participants enrolled in the programme in the course of the following two years. Twenty-eight participants of the group that never received antiretroviral therapy (never-ART) group were eligible, by CD4 cell count, for enrollment in the programme but chose not to initiate treatment. These participants did not enroll in the ART roll-out programme, probably due to the illness itself and poverty,²³ stigmatization^{23,24,25} and/or skepticism towards the conventional treatment of HIV.¹⁹ The participants not being HIV infected are the control group (uninfected group, N=154) in this sub-study.

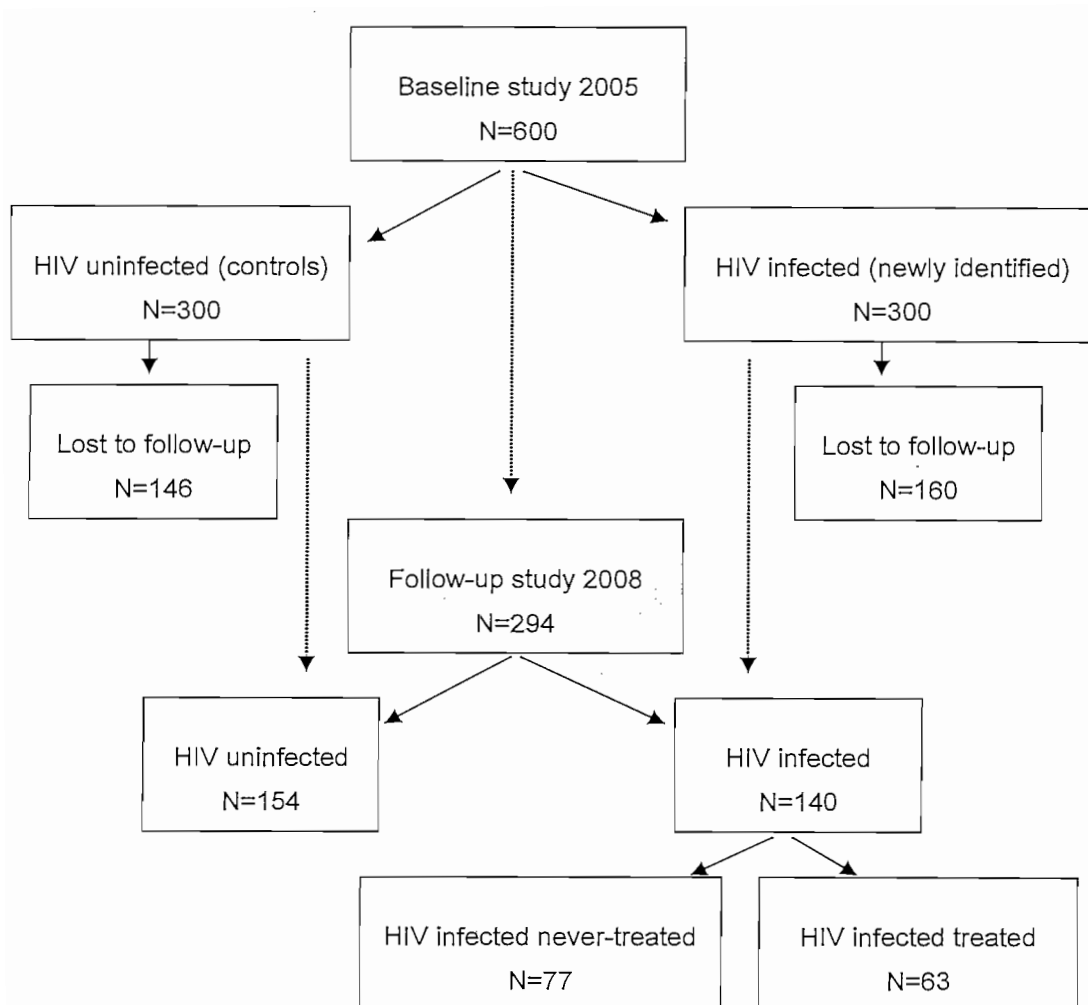


Figure 1: Outline of the study.

Ethical considerations

All participants provided signed informed consent after all procedures were explained to them in their home language. The study protocol complies with the Declaration of Helsinki as revised in 2004 and was approved by the Ethics Committee of the North-West University, Potchefstroom, South Africa.

Experimental protocol

The experimental protocol for the data collection in 2008 was identical to the data collection of 2005. To summarize - the participants arrived at the research locality of the rural or urban areas at 08:00 each morning after a 10-15 minute drive (provided by the research team) from their communities for the data collection. The participants were introduced to the setup and after the procedures were explained, they signed the informed consent forms and received HIV pre-counseling given by trained counselors. References to the local clinic or

hospital if necessary, as well as feedback on the HIV status and cardiovascular variables of the participants were given during individual post-counseling. In the course of the morning demographic and lifestyle questionnaires were completed with the help of the specially trained field workers in the subject's home language. Lifestyle data included self reported current tobacco and alcohol use as well as medical history.

Anthropometric measurements

Height, weight, hip and waist circumference (WC) were measured (Precision Health Scale, A & D Company, Japan; Invicta Stadiometer, IP 1465, UK; Holtain unstretchable metal tape) using standardized procedures.²⁶

Cardiovascular measurements

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained with the OMRON HEM-757 apparatus. After a 10 minute rest period blood pressure measurements were performed twice (5 minutes apart) on the right arm, while the participants were seated upright and relaxed with his/her right arm supported at heart level. Appropriate sized cuffs were used for obese participants.

Pulse wave velocity (PWV) was, as a measure of arterial stiffness, measured on the left side of each participant in the supine position. The measurement was determined using noninvasively accessible superficial pulses and the Complior SP device (Artech-Medical, Pantin, France) in a segment over the carotid radialis (crPWV) and carotid dorsalis pedis (cdpPWV). cdpPWV was determined only in 2008.

All carotid intima-media thickness (cIMT) measurements were performed in 2008 (not during baseline) by the same investigator using a SonoSite Micromaxx ultrasound system (SonoSite, Inc., Bothell, WA, USA) with a 6–13 MHz linear array transducer. Images from at least two optimal angles of the left and right common carotid artery were obtained. Following previously described protocols,²⁷ these segments were imaged and measured. The images were digitized and imported into the Artery Measurement Systems automated software^{28,29} for dedicated analysis of cIMT. A maximal 10 mm segment with good image quality was chosen for analysis. The programme automatically identifies the borders of the intima-media of the near and far wall, and the inner diameter of the vessel, and calculates the cIMT and diameter from around 100 discrete measurements through the 10 mm segment. This automated analysis was capable of being manually corrected if not found appropriate on visual inspection. The cross sectional wall area (CSWA) was calculated to confirm structural and not functional changes in the luminal diameter as follows: CSWA =

$\pi(d/2 + cIMT)^2 - \pi(d/2)^2$, where d denotes luminal diameter. Plaque identification was performed at the carotid bulb at each angle of the IMT measurements.

Blood, serum and plasma samples

The subjects were required to fast overnight. Blood was drawn from the antebachial vein using a sterile winged infusion set and syringes. Serum was prepared according to appropriate methods and stored at -80°C in the laboratory. In the rural area serum was stored at -18°C (no longer than five days) until it could be transported to the laboratory facility and was then stored at -80°C until analysis.

Biochemical analyses

Quantitative determination of the total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglyceride (TG), glucose and high sensitivity C-reactive protein (hs-CRP) concentration in serum were analyzed with the Konelab20iTM auto-analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland) for baseline data and with the Beckman Coulter DxC 800 Synchron[®] Clinical System (Beckman Coulter Incl., CA) for follow-up data. Low density lipoprotein cholesterol (LDL-C) was calculated by using the Friedewald formula.³⁰ Plasma (EDTA) soluble urokinase Plasminogen Activator Receptor (suPAR) levels were measured using the suPARnostic[®] ELISA kit (ViroGates, Copenhagen, Denmark). Serum concentrations of high sensitivity interleukin-6 (hsIL-6) were measured using human enzyme-linked immunosorbent assays (Quantikine[®] HS ELISA, R&D Systems, Minneapolis, USA). HIV status was determined with the First Response (PMC Medical, India) rapid HIV card test using whole blood. If tested positive, the test was repeated with the Sensa (Seyama Solutions, SA) card test. The HIV-1 subtype C epidemic prevalent in South Africa has been established by serotyping and genotyping.^{18,31,32}

Statistical analysis

All data were statistically analyzed by means of Statistica v.8 (Statsoft Inc., OK, USA, 2008). Analysis of variance (ANOVA) was used to compare the characteristics of the continuous variables of the HIV uninfected, HIV-1 infected ART and HIV-1 infected never-ART groups for the 2005 and 2008 measurements, and independent t tests when comparing the treated and never-treated HIV infected groups as well as the follow-up and lost to follow-up groups. Changes (between baseline and follow-up) within each group were determined using the t test for dependent samples reporting the mean and 95% confidence intervals. The distribution of suPAR, hsCRP and hsIL-6 were normalized by logarithmic transformation before analysis, reporting the geometric mean and the 5th and 95th percentile intervals. An analysis of covariance (ANCOVA), whilst adjusting for the baseline values of 2005, was

performed to determine differences in the percentage change in the three years between data collection. The Chi-square test was done to compare data of categorical variables, reporting number of participants and percentage of number of participants with data available. Pearson correlations were performed in the uninfected, treated and never-treated groups.

RESULTS

A comparison between and within the three groups (HIV uninfected, treated (ART) and never-treated (never-ART) group) is shown in Table 1. The ages and gender distribution of the three groups were similar. At baseline (2005), before treatment commenced, the waist circumference ($p=0.02$) and SBP ($p=0.03$) were lower and the TC:HDL-C ratio higher ($p<0.01$) in the ART group. The suPAR levels were higher in both groups of HIV infected participants compared to the uninfected participants ($p=0.03$). At the follow-up in 2008, both the TC:HDL-C ($p<0.01$) and TG:HDL-C ($p=0.02$) ratio's were higher in the never-treated group, whereas the suPAR levels were significantly higher in the ART group ($p<0.01$). After adjustments for age, gender, tobacco and alcohol use the overall results did not change.

Table 1

Comparison within and between HIV uninfected participants versus HIV infected participants (with and without treatment) after a three year follow-up period.

		HIV uninfected	<i>P</i>	HIV infected	<i>P</i>	HIV infected	<i>P</i>	<i>P</i> *
		N = 154	2005/ 2008	ART N = 63	2005/ 2008	never-ART N = 77	2005/ 2008	between groups
Age (years)	2008	48.3 ± 8.26		46.7 ± 6.46		47.8 ± 7.47		0.41
Men N (%)	2008	57 (37.0)		22 (34.9)		23 (30.2)		0.61
BMI	2005	23.5 (22.6-24.4)		21.7 (20.4-23.2)		23.6 (22.4-24.9)		0.10
(kg/m ²)	2008	23.7 (22.8-24.6)		22.0 (20.6-23.4)		23.1 (21.9-24.4)		0.15
	% Δ	1.18 (-0.14-2.50)	0.09	1.54 (-0.52-3.61)	0.58	-1.68 (-3.56-0.19)	0.02	0.03
Waist	2005	76.7 (75.1-78.3)		72.8 (70.2-75.4)		77.1 (74.8-79.4)		0.02
(cm)	2008	77.9 (76.4-79.9)		75.8 (73.4-78.9)		75.9 (73.4-78.5)		0.26
	% Δ	2.12 (0.33-3.92)	<0.01	4.68 (1.86-7.51)	<0.01	-0.72 (-3.25-1.82)	0.32	0.02
Waist:hip	2005	0.82 (0.81-0.83)		0.82 (0.79-0.83)		0.81 (0.79-0.83)		0.96
ratio	2008	0.83 (0.81-0.84)		0.84 (0.82-0.86)		0.81 (0.79-0.83)		0.23
	% Δ	1.95 (0.23-3.68)	0.10	2.7 (0.03-5.43)	<0.01	-0.03 (-2.47-2.41)	0.85	0.28
SBP	2005	130 (126-133)		121 (116-127)		129 (124-134)		0.03
(mm Hg)	2008	134 (130-137)		129 (124-134)		130 (126-135)		0.17
	% Δ	5.69 (3.65-7.73)	<0.01	5.74 (2.55-8.94)	<0.01	2.52 (-0.37-5.42)	0.58	0.18
DBP	2005	86.3 (84.0-88.6)		82.7 (79.2-86.3)		87.2 (84.0-90.4)		0.15
(mm Hg)	2008	88.6 (86.6-90.6)		86.0 (82.9-89.1)		87.7 (84.9-90.6)		0.38
	% Δ	4.63 (2.69-6.57)	0.03	4.02 (0.98-7.05)	0.04	2.64 (-0.12-5.39)	0.65	0.51
MAP	2005	101 (98.5-104)		95.6 (91.7-100)		101 (97.6-105)		0.06
(mm Hg)	2008	104 (101-106)		100 (96.8-104)		102 (98.7-105)		0.25
	% Δ	4.79 (2.96-6.64)	0.01	4.62 (1.74-7.50)	<0.01	2.42 (-0.19-5.04)	0.64	0.32
crPWV	2005	10.7 (10.3-11.0)		11.5 (10.9-12.0)		11.1 (10.6-11.6)		0.06
(m/s)	2008	11.2 (10.8-11.6)		11.8 (11.2-12.4)		11.0 (10.5-11.6)		0.14
	% Δ	6.96 (2.57-11.3)	0.02	8.81 (1.95-15.7)	0.31	1.82 (-4.40-8.04)	0.85	0.27
TC:HDL-C	2005	3.37 (3.13-3.60)		4.07 (3.69-4.44)		3.84 (3.51-4.18)		<0.01
ratio	2008	4.15 (3.77-4.53)		4.48 (3.88-5.07)		5.22 (4.69-5.75)		<0.01
	% Δ	24.6 (15.2-34.0)	<0.01	41.5 (28.6-54.4)	<0.01	23.4 (8.9-37.9)	0.07	0.08
TG:HDL-C	2005	0.90 (0.71-1.09)		1.29 (0.97-1.61)		1.16 (0.87-1.44)		0.07
ratio	2008	1.17 (0.94-1.40)		1.43 (1.12-1.84)		1.74 (1.41-2.07)		0.02
	% Δ	49.6 (31.6-67.6)	<0.01	46.1 (18.1-74.0)	0.28	83.9 (58.9-109)	<0.01	0.06
suPAR	2005	3.42 (2.11-6.11)		3.75 (2.28-7.09)		3.82 (2.36-6.57)		0.03
(ng/ml)	2008	3.46 (2.13-6.59)		4.22 (2.34-9.45)		3.86 (2.21-7.50)		<0.01
	% Δ	1.24 (-3.48-5.96)	0.64	13.8 (6.53-21.1)	0.09	4.51 (-2.10-11.1)	0.66	0.02

N indicates number of participants; BMI, body mass index; % Δ, percentage change; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; crPWV, carotid radial pulse wave velocity; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; suPAR, soluble urokinase Plasminogen Activator Receptor. Data are expressed as mean with ± standard deviation or 95% confidence intervals, N or % of N. SuPAR data are expressed as geometric mean (5th and 95th percentile intervals). *P* values 2005/2008 were obtained with dependent *t* tests, *P** values (between uninfected, treated and never-treated groups) were obtained with ANOVA, and % Δ *P* values were obtained with ANCOVA, adjusted for pre-values (2005).

When the values between 2005 (baseline) and 2008 (follow-up) were analyzed (Table 1) the WC, SBP, DBP, mean arterial pressure (MAP), and TC:HDL-C ratio increased in both the uninfected and ART groups in 2008 compared to 2005. The treated group also had a higher waist:hip ratio in 2008. None of these changes were evident for the never-treated HIV infected group; only the TG:HDL-C ratio increased in the latter group during the three years of follow-up. The uninfected group also had a higher TG:HDL-C ratio in 2008 than in 2005.

When comparing the percentage change (adjusted for baseline values) between the groups over the three years, an increase in suPAR levels ($p=0.02$), BMI ($p=0.03$) and WC ($p=0.02$) were seen in the treated participants (Table 1).

Additional characteristics of the participants including data only obtained during either baseline or follow-up are presented in Table 2. Baseline suPAR levels were lower in the HIV uninfected group (Table 1), whereas hsCRP and hsIL-6 did not differ between the groups (Table 2). Both TC ($p=0.01$) and HDL-C ($p\leq 0.01$) levels were lower in the never-treated group in 2005 and again in 2008.

Table 2

Additional characteristics of HIV uninfected, treated HIV infected (ART) and never-treated HIV infected participants.

	HIV uninfected N = 154	HIV infected ART N = 63	HIV infected never-ART N = 77	P
Baseline 2005				
hsCRP (mg/L)	2.22 (0.24-30.3)	2.64 (0.33-36.2)	2.71 (0.31-40.9)	0.55
hsIL-6 (pg/ml)	3.97 (1.15-2.28)	4.31 (1.44-17.1)	3.97 (1.18-13.9)	0.76
TC (mmol/L)	5.07 (94.85-5.27)	4.22 (3.87-4.53)	4.76 (4.44-5.03)	<0.01
HDL-C (mmol/L)	1.71 (1.61-1.82)	1.17 (1.00-1.32)	1.38 (1.23-1.51)	<0.01
LDL-C (mmol/L)	2.54 (2.39-2.69)	2.46 (2.25-2.67)	2.55 (2.32-2.78)	0.79
TG (mmol/L)	1.19 (1.05-1.31)	1.24 (1.02-1.43)	1.28 (1.09-1.45)	0.71
Glucose (mmol/L)	5.59 (5.38-5.74)	5.19 (4.91-5.47)	5.39 (5.12-5.62)	0.08
CD4 cell count (cells/mm ³)		320 (187-454)	485 (366-604)	0.07
Follow-up 2008				
TC (mmol/L)	4.25 (4.11-4.43)	4.18 (3.85-4.43)	3.76 (3.49-4.01)	0.01
HDL-C (mmol/L)	1.20 (1.12-1.28)	1.04 (0.91-1.16)	0.83 (0.72-0.94)	<0.01
LDL-C (mmol/L)	2.54 (2.39-2.67)	2.55 (2.32-2.78)	2.46 (2.25-2.67)	0.79
TG (mmol/L)	1.14 (0.99-1.27)	1.22 (1.02-1.45)	1.12 (0.92-1.32)	0.70
Glucose (mmol/L)	4.60 (4.45-4.75)	4.62 (4.39-4.85)	4.53 (4.33-4.74)	0.82
cdpPWV (m/s)	8.81 (8.54-9.07)	8.30 (7.88-8.72)	8.74 (8.36-9.12)	0.12
clMT (mm)	0.55 (0.54-0.56)	0.54 (0.52-0.55)	0.54 (0.52-0.55)	0.28
CSWA (mm ²)	11.3 (10.9-11.7)	11.1 (10.6-11.7)	11.1 (10.5-11.6)	0.63
Plaque N1 (%)	31/117 (26.5)	13/48 (27.1)	14/53 (26.4)	0.99
CD4 cell count (cells/mm ³)	-	260 (218-302)	264 (209-319)	0.91
CD4 ≤ 200 N ¹ (%)	-	17/44 (38.6)	28/57 (49.1)	0.29
Duration of ART > 2 years N (%)	-	20 (31.7)	-	-
Tobacco users N ¹ (%)	68/142 (47.9)	13/51 (25.5)	24/66 (36.4)	0.01
Alcohol users N ¹ (%)	72/142 (50.7)	16/51 (31.4)	30/66 (45.5)	0.06

N indicates number of participants; hsCRP, high sensitivity C-reactive protein; hsIL-6, high sensitivity interleukin-6; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; cdpPWV, carotid dorsalis pedis pulse wave velocity; clMT, carotid intima-media thickness; CSWA, cross sectional wall area; ART, antiretroviral therapy. Data are expressed as arithmetic mean with 95% confidence intervals, geometric mean (5th and 95th percentile intervals) for suPAR, hsCRP and hsIL-6, N or % of N. P values were obtained with ANOVA or independent t test (CD4 data). Chi-square test was used for P values of categorical variables. N¹ - data not available for all participants - N and % of participants with data available reported.

Correlations between the 2005 (baseline) levels of inflammatory markers: suPAR, hsCRP and hsIL-6 and cardiovascular and metabolic changes are shown in Table 3.

Table 3

Correlation coefficients of inflammatory markers and the percentage change of different variables of the HIV uninfected, HIV infected treated and never treated groups.

	HIV uninfected (N = 154)		HIV infected ART (N = 63)		HIV infected never-ART (N = 77)	
	log suPAR baseline	log IL-6 baseline	log suPAR baseline	log IL-6 baseline	log suPAR baseline	log IL-6 baseline
Inflammatory markers						
log hsCRP	-	r =0.39	-	r =0.40	-	r =0.47
baseline	-	p <0.01	-	p <0.01	-	p <0.01
log hslL-6	r =0.21	-	r =0.42	-	r =0.24	-
baseline	p =0.01	-	p <0.01	-	p =0.04	-
% Change 2005-2008						
BMI	-	-	r =0.32	r =0.34	r = -0.24	-
	-	-	p =0.01	p =0.01	p =0.04	-
Waist	r = -0.22	-	r =0.33	-	-	-
	p =0.01	-	p =0.01	-	-	-
TC:HDL-C ratio	-	-	r = -0.32	-	-	-
	-	-	p =0.01	-	-	-
Follow-up data 2008						
Tobacco use	r =0.32	-	r =0.31	-	r =0.28	-
	p <0.01	-	p =0.03	-	p =0.03	-
Alcohol use	r =0.26	r =0.18	-	-	r =0.28	-
	p <0.01	p =0.03	-	-	p =0.03	-

ART indicates antiretroviral therapy; N, number of participants; suPAR, soluble urokinase Plasminogen Activator Receptor; hsCRP, high sensitivity C-reactive protein; hslL-6, high sensitivity interleukin-6; BMI, body mass index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol. Only significant ($p < 0.05$) correlation coefficients given.

Inter-correlations between the baseline values of the three inflammatory markers indicated that suPAR correlated with hsIL-6, but not with hsCRP, while hsIL-6 also correlated with CRP in all three groups. In the treated group baseline suPAR correlated positively with the percentage change in BMI and waist, and negatively with the percentage change in the TC:HDL-C ratio. In the never-treated group suPAR correlated inversely with the % change in BMI and in the uninfected group negatively with the % change in WC. Correlations between suPAR and tobacco use were seen in all three groups and suPAR also correlated with alcohol use in the uninfected group and never-treated groups. hsCRP showed no correlations with changes in metabolic or cardiovascular variables. hslL-6 correlated with % change in BMI in the treated group and with alcohol in the uninfected group.

The baseline characteristics of the follow-up participants and the participants lost to follow-up are compared in Table 4. Among the HIV infected participants the crPWV, hsCRP and hslL-6 of the follow-up participants were higher than those lost to follow-up. The percentage

HIV infected men lost to follow-up were higher than those who partook in the follow-up. Among the uninfected participants the follow-up participants were older and had a higher BMI than those lost to follow-up.

Table 4

Characteristics (2005) of HIV uninfected and HIV infected participants who were lost to the follow-up and those who was part of the follow-up.

	HIV uninfected			HIV infected		
	Lost to follow-up N = 146	Follow-up N = 154	P	Lost to follow-up N = 160	Follow-up N = 140	P
Age (years)	42.8 ± 7.11	45.2 ± 8.28	<0.01	43.7 ± 8.82	44.4 ± 7.06	0.42
Men N (%)	59 (40.4%)	57 (37.0%)	0.55	71 (44.4%)	45 (32.1%)	0.03
BMI (kg/m ²)	22.2 ± 7.11	23.5 ± 8.28	0.04	22.9 ± 5.54	22.9 ± 5.68	0.95
Waist (cm)	75.1 ± 10.1	76.7 ± 9.94	0.17	76.6 ± 10.4	75.2 ± 10.8	0.24
SBP (mm Hg)	129 ± 20.6	130 ± 22.9	0.71	123 ± 23.1	125 ± 20.3	0.43
DBP (mm Hg)	85.4 ± 13.9	86.3 ± 14.7	0.59	83.1 ± 15.7	85.1 ± 13.6	0.24
MAP (mm Hg)	99.9 ± 15.5	101 ± 16.8	0.58	96.5 ± 17.6	98.6 ± 15.4	0.28
crPWV (m/s)	11.1 ± 2.21	10.7 ± 2.38	0.11	10.7 ± 2.02	11.3 ± 2.17	0.03
TC (mmol/L)	4.99 ± 1.29	5.06 ± 1.37	0.64	4.34 ± 1.23	4.51 ± 1.28	0.26
HDL-C (mmol/L)	1.69 ± 0.74	1.72 ± 0.69	0.77	1.21 ± 0.61	1.27 ± 0.54	0.36
LDL-C (mmol/L)	2.79 ± 1.06	2.82 ± 1.22	0.89	2.54 ± 1.01	2.67 ± 1.01	0.28
TG (mmol/L)	1.13 ± 0.69	1.18 ± 0.82	0.55	1.32 ± 0.76	1.27 ± 0.79	0.58
Glucose (mmol/L)	5.44 ± 1.20	5.56 ± 1.01	0.34	5.39 ± 1.32	5.29 ± 1.21	0.50
hsCRP(mg/L)	2.04 (0.22-27.4)	2.22 (0.24-30.3)	0.63	2.67 (0.32-53.1)	3.99 (0.31-40.9)	0.03
hsIL-6 (pg/ml)	3.48 (1.10-13.9)	3.97 (1.15-20.3)	0.16	4.10 (1.48-22.4)	5.29 (1.23-16.6)	<0.01

N indicates number of participants; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; crPWV, carotid radial pulse wave velocity; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; hs-CRP, high sensitivity C-reactive protein; hs-IL-6, high sensitivity interleukin 6. Data are expressed as arithmetic mean ± standard deviation, geometric mean (5-95 percentiles) or % of N. All p values were obtained with independent *t* tests, except for gender where Chi-square test was used.

DISCUSSION

After three years the HIV-1 infected participants who enrolled in the antiretroviral roll-out programme showed signs of lipodystrophy and a significantly greater increase in suPAR levels than those who did not enroll or being uninfected. The never treated HIV-1 infected participants, who are known to be HIV-1 infected for at least three years, showed no change in suPAR levels and dyslipidemia when compared to the uninfected group.

Ostrowski *et al.* found elevated plasma levels of suPAR in treatment naive HIV infected patients. However, after the introduction of HAART, a decrease in suPAR levels were seen in those who had high baseline levels of suPAR.⁴ This is in contrast to our findings, since

we found a significantly greater increase in suPAR levels ($p=0.02$) after the introduction of treatment. The baseline suPAR levels did not differ ($p=0.75$) between the two HIV-1 infected groups before the introduction of antiretroviral therapy. Although the mean baseline CD4 cell count tended to be lower ($p=0.07$) in the ART group (who started treatment after the baseline data collection), no difference ($p=0.91$) in CD4 cell count was seen between the treated and never-treated groups after three years. The latter is in agreement with the results of Ostrowski *et al.* as they concluded that circulating suPAR levels are not directly associated with immunodeficiency, but with inflammation.⁴

In view of our results the question may be asked whether elevated suPAR levels show a stronger correlation with lipodystrophy than inflammation.

According to Andersen *et al.* plasma levels of suPAR may be a marker linking immunologic, inflammatory and metabolic characteristics of HIV infected patients receiving antiretroviral therapy.¹ In their study, patients with a cluster of dysmetabolic features and lipodystrophy had relatively high levels of suPAR compared to similar patients but without lipodystrophy.¹⁵ The latter is in agreement with our study where the suPAR levels were higher in the treated (ART) group, which showed signs of lipodystrophy.

Indeed, changes in body composition were one of the most prominent results of our study. BMI decreased significantly in the never-treated HIV infected group and remained the same in both the treated HIV infected and uninfected control group. While the increase in BMI remained similar in the latter two groups, the percentage increase seen in abdominal obesity in the ART group was greater than that seen in the uninfected group. Also, the waist:hip ratio increased significantly only in the ART group, indicating clearly a abdominal fat deposition in this group. These changes in the ART group, confirm the possible development of lipodystrophy expected after the introduction of antiretroviral treatment.^{10,11}

Soluble uPAR is a good marker of low-grade inflammation in the general population³³ and according to Thuno *et al.* the strong prognostic value of suPAR may reflect a stronger linkage to immune activation compared to traditional markers of inflammation such as CRP, TNF- α and IL-6.³⁴ Furthermore, Andersen *et al.* suggested that the diurnal stable suPAR is probably more suitable as a biomarker for dysmetabolism (especially glucose tolerance and insulin sensitivity) compared to TNF- α and IL-6 in HIV infected patients on HAART.¹⁵ In our study baseline suPAR was associated with baseline hsIL-6, but not with baseline hsCRP, which is the current gold standard marker of low-grade inflammation.³⁴

However, in our normo-glycemic study population baseline suPAR correlated with three-year changes in obesity measures in the ART group who showed signs of lipodystrophy. IL-6 correlated only with changes in BMI in this group, which is in agreement with the results Andersen *et al.*¹⁵ No correlations were seen with CRP. Interestingly, correlation coefficients indicated that baseline suPAR levels in antiretroviral treated individuals correlated inversely with percentage change in TC:HDL-C ratio.

Although lipodystrophy is seen in a small portion of non-treated HIV infected individuals,³⁵ it is mostly reported in individuals with treated HIV infection,^{10,11} as was the case in our study.

Regarding the possible relation between suPAR and cardiovascular disease, Pawlack *et al.* found high suPAR levels were independently related to cIMT in uremic patients.¹⁷ Also, uPAR was found to be significantly higher in the intima of atherosclerotic lesions.³⁶ Although the suPAR levels were higher in our ART group, no significant differences were seen in blood pressure, cIMT, cross sectional wall area, plaque prevalence or arterial stiffness between the treated, never-treated and uninfected groups in 2008. These results, therefore, do not seem to indicate an increased risk for cardiovascular disease characterized by atherosclerosis, structural (IMT) or functional (PWV), in any of the three groups. A three year follow-up is perhaps too short to observe more significant changes.

Cigarette smoking is of particular concern among HIV infected individuals given the prevalence of insulin resistance, dyslipidemia, abdominal obesity and cardiovascular disease among HIV infected persons in the HAART era.¹⁰ Although the tobacco use was less in our treated group, it correlated significantly and positively with suPAR in all three groups. This is in agreement with the study of Eugen-Olsen *et al.* where suPAR was increased by smoking in the general population.³³ The significant correlation of suPAR with alcohol use in the uninfected and never-treated groups also indicates that lifestyle habits may impact on suPAR levels.

This study should be interpreted within the context of its strengths and limitations. Although many participants were lost during the follow-up, the study still had sufficient power after three years. A comparison between those lost to follow-up and the follow-up participants in Table 4 indicates a few differences; both in the HIV infected and uninfected participants. The HIV-1 infected participants who partook in the follow-up showed higher levels of inflammatory markers and a higher pulse wave velocity. Thus, those with a worse health profile participated in the follow-up.

Our study population is unique as we studied black Africans who qualified for the community-based roll-out programme for HIV infected individuals. The infected individuals were infected for at least 3 years with the HIV-1 in South Africa, where, most likely, the subtype C virus is prevalent and treatment became freely available only in 2004. A limitation of the study is that the participants were started on ART during the course of the three years of the study and that all participants did not use ART for the full three-year period. Unfortunately cIMT measurements were not performed during baseline data collection, thus the probable progression in cIMT could not be assessed. We have no information regarding kidney function or the presence of insulin resistance. We were unable to test for tuberculosis or other opportunistic infections, but the participants perceived themselves as healthy when they enrolled to the study. Our results may thus have been confounded by non-measured factors, but the prospective nature of this study could assist in this regard. Due to the longitudinal nature of this study, we plan to collect this information in future.

In conclusion, this study shows that HIV-1 infected black South Africans have significantly higher suPAR levels than uninfected controls. Furthermore, treated HIV-1 infected Africans show a significant larger increase in blood suPAR levels than never-treated infected or uninfected Africans after a three year period. Baseline suPAR correlated positively with abdominal obesity and inversely with the % change in TC:HDL-C ratio in the treated HIV-1 infected Africans. No significant correlation of suPAR with cardiovascular variables was seen, but baseline suPAR correlated with tobacco use in all three groups of participants. This study indicates a association of suPAR with the development of lipodystrophy in HIV-1 infected black South Africans on the WHO's recommended first line antiretroviral therapy.

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CHAPTER 5

FINDINGS AND CONCLUSIONS

FINDINGS AND CONCLUSIONS

INTRODUCTION

In this chapter, a summary of the main findings from the three manuscripts reported in this thesis will be given. The results of each manuscript will be discussed, interpreted, elucidated and compared to the relevant literature. Conclusions will be drawn, and recommendations will be made regarding HIV-1 and the development of cardiovascular disease in black South Africans.

SUMMARY OF MAIN FINDINGS

The findings of the three manuscripts reported in this thesis are:

1. **TITLE:** LIPID ABNORMALITIES IN A NEVER TREATED HIV-1 SUBTYPE C INFECTED AFRICAN POPULATION

The aim of this first manuscript was to evaluate whether dyslipidemia and an increased incidence of the metabolic syndrome were seen in our never-treated newly identified HIV-1 infected participants. It was hypothesized that these participants would show dyslipidemia compared to their uninfected controls, but that this would not increase the prevalence of the metabolic syndrome in this population.

The results indicated that HIV-1, specifically subtype C, is associated with dyslipidemia and an inflammatory state in HIV infected African individuals that may increase their risk for cardiovascular disease. The study shows that HIV-1 subtype C, though genetically different from subtype B, seems to influence the metabolic syndrome components in the same way as HIV-1 subtype B, but does not increase the prevalence of the metabolic syndrome in Africans.

2. **TITLE:** IS HIV-1 ASSOCIATED WITH ENDOTHELIAL DYSFUNCTION IN A POPULATION OF AFRICAN ANCESTRY IN SOUTH AFRICA?

The aim of the second manuscript was to assess whether our newly identified, never-treated, HIV-1 infected participants show signs of endothelial dysfunction, accelerated atherosclerosis and increased coagulation. We hypothesized that these participants would show signs of endothelial injury, endothelial dysfunction and increased coagulation.

The results showed lower HDL-C and higher IL-6, CRP, ICAM-1 and VCAM-1 levels indicating endothelial injury in the HIV-1 infected participants compared to the individually matched uninfected controls, but no signs of increased coagulation were detected. However, a continuous positive trend of increasing peripheral arterial stiffness

with increasing age, indicative of early vascular aging, was detected in the HIV-1 infected Africans.

3. **TITLE:** SuPAR IS ASSOCIATED WITH METABOLIC CHANGES IN HIV-1 INFECTED AFRICANS THREE YEARS AFTER ENROLLING FOR TREATMENT

In this last manuscript the aim was to determine whether suPAR levels are elevated in HIV-1 infected black South Africans, treated and never-treated, compared to uninfected controls before and after a three year follow-up. The second aim was to investigate whether baseline suPAR levels are associated with similar cardiovascular and metabolic changes over the three year period in the HIV-1 infected individuals, treated and never-treated. We hypothesized that the suPAR levels are increased in HIV-1 (most likely subtype C) infected participants and decreased in treated infected participants after a three year period. We further hypothesized that antiretroviral treatment stabilizes the lipid profile of HIV-1 infected participants, but these participants would show an increase in cardiovascular variables associated with cardiovascular disease.

This study showed that HIV-1 infected black South Africans had significantly higher suPAR levels than uninfected controls. However, the main result of this manuscript was that treated HIV-1 infected participants showed signs of lipodystrophy and a greater increase in suPAR levels compared to the never-treated and uninfected participants after three years. In the treated HIV-1 infected group suPAR levels were associated with an increased waist circumference and correlated inversely with TC:HDL-C ratio. No associations were seen with either baseline CRP or IL-6. The never treated HIV infected participants showed no increase in suPAR levels in the three year time-span and a worse lipid profile when compared to the uninfected control participants.

A COMPARISON OF FINDINGS WITH THE LITERATURE

When the results of this study, as found in HIV-1, most likely subtype C, infected participants, are compared with the results found in the literature regarding mostly subtype B HIV-1 infection in other population groups, it is evident that most findings were confirmed, while some others contradicted those found in the literature.

The dyslipidemia and inflammation as seen in the HIV-1, most likely subtype C, infected participants are confirmed by the literature^{1,2,3} where HIV-1 (subtype B) prevails.⁴ The low HDL-C and higher TG levels, exhibited by the HIV-1 infected participants, confirmed results seen in the SIMONE study where the diagnosis of the metabolic syndrome in HIV infected individuals was mainly due to reduced HDL-C and high serum TG levels.⁵

Much controversy still exists whether HIV infected individuals have a higher prevalence of the metabolic syndrome (with or without therapy). The prevalence of the metabolic syndrome of 15% (ATP III definition) and 21% (IDF definition) in our never-treated HIV-1 infected participants is in agreement with the study by Samaras *et al.* in treated HIV-1 infected individuals.⁶ In our never-treated HIV infected participants the prevalence of the metabolic syndrome (both according to the ATP III and IDF definition) did not differ between the HIV infected and the age, gender, body mass index and locality matched uninfected controls. This finding is in contrast with the finding of Bonfanti *et al.* who reported a higher prevalence of the metabolic syndrome in never-treated HIV infected individuals than in the general population.⁷ It is not clear why our results are different from those of Bonfanti *et al.*, but perhaps the different virus subtype, ethnic groups and socioeconomic status might account for the differences. The duration of the HIV-1 infection is unknown in our infected participants and this might also have influenced the prevalence of the metabolic syndrome.

Although lipodystrophy is seen in a small portion of non-treated HIV infected individuals,⁸ it is mostly reported in individuals with treated HIV infection.^{9,10} This research, although done within an ethnic different population group where another strain of HIV-1 prevails, confirms the latter where our results showed the development of lipodystrophy after three years in the treated participants. The development of lipodystrophy in our treated participants is further confirmed by that found in the literature where stavudine, the NRTI of the first line therapy of the roll-out programme, is incriminated in the development lipodystrophy.¹¹

Our results partly confirm the suggestion of Andersen *et al.* that suPAR is more suitable as a biomarker for dysmetabolism (especially glucose tolerance and insulin sensitivity) compared to IL-6 and CRP in treated HIV infected patients, as baseline suPAR was associated with lipodystrophy, while no associations were seen with either baseline IL-6 or CRP.¹² However, our participants were normo-glycemic and our results indicated that baseline suPAR levels in treated individuals correlate inversely with the percentage change in TC:HDL-C ratio and this novel result have not been reported in the literature. It, therefore, seems that our results rather confirm the association between suPAR and lipodystrophy,¹² and not between suPAR and dysmetabolism. Although the literature does not report the association between suPAR and an improvement in lipids in treated HIV infected individuals, increases in HDL-C levels with the first line therapy have been reported.¹³⁻¹⁵ The inverse association between baseline suPAR and percentage change in TC:HDL-C ratio in our treated group could therefore be due to the treatment rather than possible beneficial effects of suPAR.

Increased levels of the inflammatory markers CRP, IL-6¹⁶ and cell adhesion molecules, ICAM-1 and VCAM-1,¹⁷ have been reported in the HIV infected population.^{2,18} Our results confirm the latter in HIV-1 infected black Africans. In our never-treated participants we confirmed the inverse relationship between HDL-C and IL-6.¹⁹ In contrast to that found in the literature²⁰ our results showed an increase in suPAR levels after three years in the treated group, indicating that suPAR may not reflect a stronger linkage to immune activation compared to traditional markers of inflammation such as CRP and IL-6 as suggested by Thuno *et al.*²¹ It rather confirms the conclusion of Ostrowski *et al.* that circulating suPAR levels are not directly associated with immunodeficiency, but with inflammation.²⁰

The SBP was lower in the never-treated HIV infected group, thus confirming in the literature.²² After three years both SBP and DBP increased in the uninfected and treated groups, while no change was seen in the never-treated group. Although our results did not indicate hypertension in the treated participants, the increase in blood pressure could indicate the development of hypertension which then could confirm the association between hypertension and antiretroviral therapy found in the HIV infected population.²³

Kidney function is often compromised in the HIV infected population, and HIV-associated nephropathy is frequently seen in immunosuppressed individuals of black ethnicity.²⁴ In contrast, no difference in serum creatinine or estimated creatinine clearance rate was seen between the HIV infected and uninfected participants in our study. The lack of indications of compromised kidney function may be related to the most likely short duration of the of the HIV-1 infection in our study population as they were newly identified as being HIV infected.

Studies from the literature showed increased central aortic stiffness in treated²⁵ and untreated²⁶ HIV infected individuals. Central stiffness was not determined in our study, but we showed no difference in peripheral arterial stiffness between the HIV infected participants compared to their controls. Our results may, however, point to premature vascular aging in the HIV infected participants. Although this was found in never-treated HIV-1 infected individuals, it confirms the results of Lorenz *et al.* who obtained a higher “vascular” age of 4-5 years for treated HIV infected patients compared to controls.²⁷ Our results might therefore indicate vascular deterioration in HIV infected Africans without therapy.

In our study neither the PAI-1 activity nor fibrinogen were increased in the HIV infected subjects in contrast to that found by other researchers, although their studies were done on HIV infected individuals (75% were on HAART) in other population groups.^{17,28,29} These findings are, however, in agreement with the findings of James *et al.* who found that HIV

infection was not associated with fibrinogen concentration in never-treated HIV-1 infected Africans.³⁰ The most likely short duration of the HIV infection might explain the lack of an increased coagulation found in our never-treated HIV-1 infected Africans.

DISCUSSION OF MAIN FINDINGS

The main focus of this study was directed at the influence of various aspects of HIV on vascular function and probable increased risk for cardiovascular disease. HIV infection paradoxically affects cardiovascular risk factors and circulatory disease within populations and individuals. The literature have shown that HIV infection, and especially the use of ART, increases the risk for cardiovascular disease³¹ and are associated with an increase in insulin resistance, dyslipidemia,³² lipodystrophy^{9,33} endothelial dysfunction,¹⁷ accelerated atherosclerosis¹ and coagulation disorders.³⁴

Dyslipidemia

The results of this study showed that HIV-1, most likely subtype C, is associated with dyslipidemia but not with a higher incidence of the metabolic syndrome in never-treated individuals. After being infected for at least three years, the dyslipidemia worsened in the never-treated participants, but improved in the treated participants. The HDL-C levels exhibited by our HIV-1 infected population during the follow-up were lower (both in the never-treated and treated groups) than the levels seen as an increased risk for cardiovascular disease in the Caucasian population.³⁵ Foulkes *et al.* found that black patients on antiretroviral treatment had a less atherogenic lipid profile compared to Caucasians and Hispanics.³⁶ Their data is consistent with epidemiological data in uninfected populations, but may be of particular importance in South Africa's HIV-1 infected population. Race/ethnicity is often considered a surrogate for environmental influences on lipids, but recent studies demonstrate that genetic factors may also account for important differences in plasma lipids across ethnic groups.³⁶

Lipodystrophy

Changes in body composition were some of the most prominent results of the three year follow-up study. BMI decreased significantly in the never-treated HIV infected group and remained the same in both the treated HIV infected and uninfected control group. While the increase in BMI remained similar in the latter two groups, the percentage increase seen in abdominal obesity in the treated group was greater than that seen in the uninfected group. Also, the waist:hip ratio increased significantly only in the ART group, indicating clearly an abdominal fat deposition in this group. These changes indicate the development of lipodystrophy in the treated group. The independent association of baseline suPAR with

abdominal obesity and with an improved lipid profile in the treated HIV-1 infected Africans was another significant result in the follow-up study. This result could indicate that suPAR is associated with lipodystrophy and probable low-grade inflammation rather than dyslipidemia in our African population. The unexpected result of a greater increase in suPAR levels in the treated HIV-1 infected participants is difficult to explain. Increased inflammation, measured as elevated suPAR levels, may be a driver of lipodystrophy development and/or the lipodystrophy development may increase the inflammation with a consequently increase in suPAR levels. This might be an indication of positive feedback.

Inflammation and vascular function

During the baseline study the never-treated participants showed higher levels of inflammatory markers (CRP and IL-6) and the adhesion molecules (ICAM and VCAM), indicating endothelial injury. The higher levels of inflammatory markers and low HDL-C levels could point to endothelial dysfunction which is seen as the link between infection, inflammation and atherosclerosis.³⁷ Although endothelial dysfunction was not indicated by the PWV measurements, and IMT measurements were not done during the baseline study, a positive trend of increasing peripheral PWV with increasing age was detected, which was not observed in the uninfected participants. This could indicate accelerated vascular aging in the HIV infected population. Thirty six percent of the never-treated participants of the follow-up study were eligible, by CD4 cell count, to antiretroviral treatment. These participants did not enrol in the programme, and, therefore, did not receive treatment, probably due to the illness itself and poverty,³⁸ stigmatization³⁸⁻⁴⁰ and/or scepticism towards the conventional treatment of HIV.⁴¹ In South Africa the prevalence of atherosclerotic disease, historically not common in most black Africans, is increasing.⁴² It is possible that this vascular aging, seen in the never-treated participants could play a role in the increasing prevalence of atherosclerotic disease in black South Africans, as a substantial unmet need for treatment remains in adults.⁴³

Three years after the baseline measurements, no indication of vascular deterioration (either functional or structural endothelial dysfunction) was detected in both the treated or never-treated participants. During the baseline measurements no difference in coagulation factors were detected between the never-treated infected and uninfected groups. A three year follow-up is perhaps too short to observe more significant changes and give a picture of the influence of HIV-1 on vascular function and the development of future atherosclerosis in the HIV infected population.

CONCLUSIONS

The conclusions of the study could be summarised as follows:

- Our results show lower systolic blood pressure and dyslipidemia in the HIV-1 (most likely subtype C) infected participants in accordance to the hypothesis. An increase in systolic blood pressure, but no hypertension, and an improvement in lipid profile were seen in the treated HIV-1 infected participants. Although the HDL levels were lower and triglyceride levels higher in the HIV-infected participants, this did not increase the prevalence of the metabolic syndrome in these participants. These results do not show an influence of HIV-1, most likely subtype C, infection on the vascular function of the participants. However, the influence of the low HDL-C and high triglyceride levels on the inflammation and probable development of endothelial dysfunction and accelerated atherosclerosis, in especially the never-treated HIV infected population, should not be disregarded. The high levels of protective HDL-C in black South Africans is thought to be the reason for the fairly low prevalence of ischemic heart disease in the general population.⁴⁴ Furthermore, the antiretroviral treatment stabilized the lipid profile, but increased lipodystrophy may influence the development of future cardiovascular disease.
- We hypothesized that endothelial dysfunction, accelerated atherosclerosis and increased coagulation would be observed in the HIV-1 infected population. The findings of the study suggest inflammatory injury of the endothelium, pointing to endothelial dysfunction of never-treated, HIV-1 infected South Africans. Although the increase in atherosclerotic biological markers (ICAM and VCAM) were shown in the second manuscript, no indication of functional or structural endothelial dysfunction or atherosclerosis could be detected after three years of being HIV-1 infected in either treated or never-treated participants. No indication of increased coagulation, which could result in atherosclerotic disease, could be detected which contradicts our hypothesis. There is, however, an indication of accelerated vascular aging (PWV vs. age) and probable early atherosclerosis in the older HIV infected participants. The latter indicates a decrease in vascular function of the never-treated HIV-1 infected older population.
- We also hypothesized that the HIV-1 infected black South Africans would have significantly higher suPAR levels than their uninfected controls. This was confirmed by our results, however, the treated HIV-1 infected Africans showed a significantly greater increase in blood suPAR levels than never-treated infected or uninfected Africans after a three year period. Furthermore, this study indicates an association of suPAR with the development of lipodystrophy in normo-glycemic HIV-1 infected black South Africans on the WHO's recommended first line antiretroviral therapy.

- Poor lifestyle habits such as tobacco and alcohol use amongst the HIV infected population may contribute significantly to the risk of the development of cardiovascular disease in black South Africans.

In the past 15 years South Africa has seen a rise in non-communicable diseases which is predicted to increase in the next decades if measures are not taken to combat the trend.⁴⁵ This rise in non-communicable disease, such as cardiovascular disease, is masked by the overwhelming presence of communicable diseases like HIV and tuberculosis.⁴⁶ The results of this study are, therefore, valuable in contributing to the current knowledge regarding the probable influence of HIV-1, most likely subtype C, on vascular function and future cardiovascular risk amongst the HIV infected population in South Africa.

The South African HIV population has access to free antiretroviral treatment only since 2004 and the influence of antiretroviral treatment on the cardiovascular system in this population is not yet established. Even so, the South African National AIDS Council updated the HIV treatment guidelines and adopted some of the recent recommendations made by the World Health Organization. The new guidelines which came into effect on 1 April 2010 phase out the use of stavudine (incriminated in the development of lipodystrophy) in favour of tenofovir and permit earlier treatment for pregnant women and people with HIV and tuberculosis. According to the Council, this change will ensure that South Africa will have 1 million people on treatment by the end of June 2010.⁴⁷ This expansion of the antiretroviral therapy and the effect thereof on the burden of non-communicable diseases (as cardiovascular diseases) in South Africa is uncertain. Thus, the influence of HIV-1 on vascular function, how it influences the risk of cardiovascular disease in HIV infected South Africans and how the influence is affected by the antiretroviral treatment of the roll-out programme remains largely unknown. Longer-term research should assess the contribution of these HIV- and antiretroviral treatment-induced changes to vascular function and cardiovascular disease risk. At present the various effects of the virus itself – including its cardiovascular effects – is just as important, since many HIV infected South Africans still are without therapy and/or unaware of their infected status.

Due to the longitudinal design of this study, it could give direction for future research and could be used for recommendations to health professionals and policy makers.

CHANCE AND CONFOUNDING

The results and conclusions of the study led to recommendations, but before the recommendations are formulated, it is important to reflect critically on some factors that may have confounded the results.

Firstly, the duration of the HIV infection is unknown as the participants were newly identified as being HIV infected during the baseline study. The lack of elevated fibrinogen and PAI-1 levels in the infected group in the baseline study may be related to the most likely short duration of the infection. Also, for the baseline study only few CD4 cell counts were available as some of the participants chose not to visit the local clinic or hospital for follow-up (CD4 cell count determination and possible subsequent treatment) after they were informed of their HIV infected status. Furthermore, the participants of the follow-up study commenced on treatment during the course of the study when their CD4 counts dropped below 200 cells/mm³. The exact duration of the treatment is therefore uncertain.

Many the participants were lost to the study during the follow-up after three years. Migration of some of the participants to seek work elsewhere (as job opportunities are scarce), might have influenced the turnout of the follow-up study. Black South African individuals are often sceptical towards research, especially if it does not result in direct health benefits. There is also still a lack of knowledge regarding HIV infection and AIDS. The participants are especially unwilling to take part in research studies when blood samples are taken and HIV testing is involved. Although many participants were lost to follow-up, the follow-up study still had sufficient power. It is also interesting to note that the HIV-1 infected participants who partook in the follow-up study had a worse health profile than those lost to follow-up.

Methodological issues could also have weakened the study. Peripheral (carotid-pedalis) and not central (carotid-femoralis) PWV were performed. IMT measurements were only performed during the follow-up study three years after baseline, and not during baseline. Tuberculosis or other opportunistic infections (the participants perceived themselves as healthy) were not tested for, and there is no information regarding the presence of insulin resistance. The results may thus have been confounded by non-measured factors, but the prospective nature of this study could assist in this regard, especially since future follow-up studies are scheduled for the next seven years.

Because the contribution of HIV to cardiovascular variables is difficult to distinguish from those of classic cardiovascular risk factors, control participants were carefully matched by gender, age, BMI and locality to minimize the confounding effects of these non HIV related

conditions on the results of this study. Due to the loss of participants after three years to the follow-up, adjustments for age and gender were made in the third manuscript. The latter adjustments did not change the overall results. In all three manuscripts adjustments were made for confounders such as tobacco use and alcohol intake.

RECOMMENDATIONS

Formulation of the recommendations are aimed to assist health professionals, policy makers and health departments of the government of South Africa to gain knowledge regarding the possibility of an increase in cardiovascular disease amongst the HIV infected population of South Africa.

The following recommendations are formulated:

- Diabetes and insulin resistance are increasing health problems in South Africa. Although the prevalence of the metabolic syndrome does not seem to be increased in the never-treated HIV-1 infected population, the influence of the treatment on the prevalence of diabetes, insulin resistance and the metabolic syndrome needs to be established.
- The vascular deterioration seen in the older HIV infected population needs to be studied further. The influence of the antiretroviral therapy on vascular function needs to be determined. Especially as examination of the leading causes of death in men and women aged 50-64 years and older draws attention to the rise in deaths from vascular disease.⁴⁸
- The high prevalence of tobacco use and alcohol intake, thus lifestyle habits, amongst the South African population, also the HIV-1 infected population, should be addressed as it could have a substantial influence on the development of cardiovascular disease.
- Education on cardiovascular disease and the risk involved, as well as promotion of a healthier lifestyle is needed amongst the HIV infected population of South Africa.
- Our study underscore the need for future studies and longer term follow-up studies, addressing the metabolic and cardiovascular complications of the first line treatment of the roll-out programme that specifically account for ethnic heterogeneity and HIV-1 subtype variants.

FINAL REMARKS

The high infection rate and overwhelming prevalence of HIV infection in South Africa were mentioned several times in the thesis. Various factors seem to contribute to the negative effects of HIV in South Africa, such as the lack of knowledge, poverty, stigma, scepticism and lack of interest. Subsequently, this seems to have an impact on economic level where HIV infection and the treatment thereof place an enormous burden on government funds and

the delivery of health-care services. It also has an impact on the health of the South African population as our results (and others) clearly indicate a detrimental health profile in those infected (whether receiving treatment or not).

A national HIV counselling and testing campaign was launched on 20 April 2010 aiming to mobilise people to know their HIV status, support people with key prevention interventions to take proactive steps to a healthy lifestyle irrespective of HIV status, increase the incidence of health seeking behaviour, and increase access to treatment, care and support services.⁴⁹ This campaign hope to test 15 million people by the end of June 2011, aiming to reduce the negative perceptions and stigma surrounding the disease.⁴⁹

One of South Africa's key challenges is prevention of the spreading of HIV. The best hope of ultimately controlling the devastating epidemic is to keep trying to find a vaccine. Recently, Dr. Alan Bernstein, executive director of Global HIV Vaccine Enterprise said "A vaccine is possible, and we have the scientific tools now to turn that possibility to reality." Some researchers are however sceptical and predict the expected success to take at least another 15 years.⁵⁰ While waiting for the above initiatives to take effect, it remains of the utmost importance to gain knowledge about the influence of HIV infection on cardiovascular risk of the South African population.

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