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Acute coronary syndromes in black South African patients with human immunodeficiency virus infection

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ACUTE CORONARY SYNDROMES IN BLACK SOUTH AFRICAN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Anthony Charles Becker

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg

in fulfilment of the requirements for the degree of

Doctor of Philosophy

Johannesburg 2011

DECLARATION

I, Anthony Charles Becker declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

_____

Anthony Charles Becker Date:

I certify that the studies contained in this thesis have the approval of the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg.
Human Research Ethics Committee protocol number: **M040702**

_____

Anthony Becker (Candidate) Date:

_____

Karen Sliwa (Supervisor) Date:

DEDICATION

To my wife Tina and children, Michael and Keila, whom I love with all my heart: thanks for affording me the opportunity to complete this thesis. Without your love and support this would not have been possible.

To my parents who have always given me unconditional love and support, thank you both.

PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS BY THE CANDIDATE INCLUDED IN THE THESIS

Chapter Three

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Chapter Four

Amira C, Ntyintyane L, Wilkinson D, Stewart S, Becker A, Libhaber E, Sliwa K. Emerging epidemic of cardiovascular disease among urban Africans: Acute coronary syndrome at Baragwanath Hospital, Soweto. ***SA Heart*** **Spring 2006**

Chapter Five

Becker A, Sliwa K, Stewart S, Libhaber E, Essop AR, Zambakides C, Essop MR. Acute coronary syndromes in treatment-naïve black South African patients with Human Immunodeficiency Virus infection. ***Journal of Interventional Cardiology*** **2010; 23:70-77**

Chapter Six

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Clinical and Applied Thrombosis/Haemostasis first published on **May 11 2010**.

Chapter Seven

Becker A, Libhaber E, Sliwa K, Stewart S, Tikly M, Essop MR. Antiphospholipid antibodies in black South Africans with HIV and acute coronary syndromes: Prevalence and clinical correlates. Submitted to ***BMC Research Notes*** **October 2010**

Chapter Eight

Becker A, Libhaber E, Sliwa K, Stewart S, Essop MR. Markers of inflammation and endothelial activation in Black South Africans with HIV and acute coronary syndromes.

Journal of AIDS and HIV Research **September 2010; 2:95-100**

LIST OF PRESENTATIONS

PEER-REVIEWED NATIONAL AND INTERNATIONAL CONFERENCE ABSTRACT PRESENTATIONS BY THE CANDIDATE RELATING TO PUBLICATIONS INCLUDED IN THE THESIS.

11th Annual Congress Of The South African Heart Association, 8-11 August 2010: Oral Presentation "Acute Coronary Syndromes In Black South African Patients With Human Immunodeficiency Virus Infection: The Role Of Antiphospholipid Antibodies And Markers Of Inflammation / Endothelial Activation ."

South African Heart Association silver prize for best oral presentation

10th Annual Congress of the South African Heart Association, 22-25 October 2009: Oral Presentation "HIV, atherothrombosis and acute coronary syndromes"

15th Congress of the Southern African Hypertension Society

25-28 May 2007: Poster presentation "Acute coronary syndromes in Black South African patients with Human Immunodeficiency Virus infection: Initial results of the clinical and angiographic features."

Lionel Opie award for best poster presentation

EuroPCR, Barcelona, faculty guest, 22-25 May 2007:

Oral presentation "Coronary artery disease and PCI in HIV patients"

ABSTRACT

Background: South Africa is considered to be a country in epidemiologic transition with increasing rates of cardiovascular disease. In addition, it faces an HIV pandemic, with an estimated 5.5 million people infected and five hundred thousand HIV-related deaths annually. Current evidence suggests that patients infected with HIV are at a heightened risk for acute coronary syndromes (ACS) related to traditional cardiovascular risk factors, as well as factors related to the virus and its treatment (highly active anti-retroviral therapy (HAART)). HIV infection itself may independently predispose to coronary artery disease (CAD) by promoting endothelial dysfunction, a heightened pro-inflammatory state, dyslipidaemia and thrombosis, the aetiology of which is thought to be multifactoral in nature.

Protease inhibitor (PI) therapy, as part of HAART, has the potential to induce an adverse metabolic phenotype, including: dyslipidaemia, insulin resistance, endothelial dysfunction and a prothrombotic state. The attributable risk of these factors in HIV-associated CAD and ACS is currently unknown, but it seems that the risk of ACS is increased by prolonged exposure to PI's. No data currently exists on CAD in HIV patients not receiving HAART, which is problematic considering that this makes up the majority of patients in sub-Saharan Africa and that the combination of epidemiologic transition and HIV infection has the potential for greater cardiovascular morbidity, particularly with respect to atherothrombotic events.

Aims: The aims of this thesis are twofold. Firstly, to confirm reports of epidemiologic transition in South Africa from a broad epidemiological perspective. Secondly, by focusing on treatment-naïve HIV positive black South Africans with ACS, it aims to determine differences compared to HIV negative patients with respect to demographics and risk factors, angiographic and treatment related factors as well as markers of thrombosis and inflammation with a view to providing more focused primary and secondary prevention.

Methods: All the studies contained in this thesis were conducted in the Department of Cardiology, Chris Hani Baragwanath Hospital and adhere to the declaration of Helsinki. The first of the epidemiological studies, The Heart of Soweto (HOS) study (Chapter 3), was a prospectively designed registry that recorded epidemiologic data relating to the presentation, investigations and treatment of 1593 patients from Soweto with newly diagnosed cardiovascular disease during the year 2006. The second study (Chapter 4) was a cross sectional study of patients with ACS admitted to the Baragwanath coronary care unit over the year 2004 compared to the years 1975-1980.

The HIV sub-study (chapters 5-8) was a prospective single centre study conducted from March 2004 to February 2008. During this time, 30 consecutive black HIV patients presenting with ACS (ACS+: HIV+ group) were enrolled. For each HIV patient with ACS, the first presenting non-HIV black patient with ACS was selected as a case control comparator (ACS+ : HIV- group). In addition, a second control group of 30 asymptomatic HIV patients, who were matched for age, sex and ethnicity (ACS- : HIV+ group), were recruited from the HIV clinic. The methodology used to compare the groups involved: clinical and demographic data collection, routine blood test evaluation, angiographic

analysis and specific laboratory testing of various research blood parameters (including thrombotic screening and markers of inflammation and endothelial activation).

Results: Chapter 3 presents the results of the large HOS study, which showed good evidence to support the theory of epidemiologic transition in Soweto. Adding to this data are the results of Chapter 4, which clearly demonstrate a substantial increase in the number of patients diagnosed with ACS at Baragwanath in recent years. Consistent with a population in epidemiologic transition, there was more than a ten-fold increase in the rate of coronary events over two decades, paralleled by increased rates of modifiable risk factors. Chapter 5 presents the clinical and angiographic data from the HIV sub-study.

HIV patients with ACS were younger and had fewer traditional risk factors for CAD except for higher rates of smoking and lower HDL cholesterol levels. HIV patients had less atherosclerotic burden angiographically, but a higher thrombus burden in the infarct related arteries, suggesting a possible prothrombotic state. In addition, HIV patients had higher rates of in-stent restenosis of bare metal coronary stents at follow up. Chapters 6 and 7 present data on the thrombotic parameters between the groups, with Chapter 6 focusing mainly on coagulation pathways and Chapter 7 focusing on antiphospholipid antibodies (aPL). Chapter 8 presents data on levels of pro-inflammatory cytokines and endothelial activation markers. Greater evidence of thrombophilia was found in HIV patients with ACS as evidenced by lower Protein C (PC) levels, higher levels of Factor VIII and a higher inflammatory burden with greater degrees of endothelial cell activation - all of which increase thrombotic risk. Antiphospholipid antibodies were more prevalent in HIV patients but did not seem to be causal in the pathogenesis of thrombosis.

Conclusion: Soweto, a large, predominantly black urban area in South Africa, is in a state of epidemiologic transition, with an increasing prevalence of modifiable cardiovascular risk factors and ischaemic heart disease. Treatment-naïve HIV positive black patients presenting with ACS have different clinical and angiographic features compared to the HIV negative population. The patients are younger, more commonly male, with high rates of smoking, lower HDL levels and less atherosclerotic burden. However, there is a higher thrombotic burden, suggesting a prothrombotic state, which was evident by lower PC levels, higher factor VIII levels with a higher inflammatory burden and a greater degree of endothelial cell activation – all factors associated with a pro-atherogenic and prothrombotic state. The exact pathogenic role of HIV, independent of associated modifiable and non-modifiable risk factors, is difficult to determine, but may be important as a contributory factor in an already "vulnerable" patient. Importantly, we identified modifiable risk factors in the HIV group. Smoking may play a crucial role in the pathogenesis of ACS in these otherwise seemingly low risk patients and remains an important target for cardiovascular risk reduction.

The role of HDL in the pathogenesis and prevention of HIV-associated CAD needs to be further defined, as does the role of drug eluting coronary stents in the prevention of in-stent restenosis. Cardiovascular risk assessment and appropriate primary prevention should be an important component in the management of HIV patients, regardless of treatment status. With the anticipated increase in CVD in South Africa, further research projects appropriate to the South African context will be vital in order to explore cost effective ways to provide primary and secondary prevention in order to effectively deal with the burden of epidemiological transition as well as the cardiovascular burden likely to be imposed by the HIV pandemic.

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LIST OF ABBREVIATIONS

AC:	abdominal circumference
ACL:	automated coagulometer
aCL:	anticardiolipin antibody
ACS:	acute coronary syndrome
ADP:	adenosine diphosphate
AMI:	acute myocardial infarction
anti-β2-GPI:	anti-β2-glycoprotein 1 antibody
APC:	activated protein C
APCR:	activated protein C resistance
aPL:	antiphospholipid antibody
aPPT:	activated partial thromboplastin time
aPT:	antiprothrombin antibody
APS:	antiphospholipid syndrome
ARC:	academic research consortium
ART:	antiretroviral therapy
ARV:	antiretroviral
AT:	antithrombin
AIDS:	acquired immune deficiency syndrome
BMI:	body mass index
BMS:	bare metal stent
$CaCl_{2:}$:	calcium chloride

CAD:	coronary artery disease
CAMS:	cellular adhesion molecules
CCU:	coronary care unit
CDC:	centre for disease control
CD4:	cluster of differentiation 4
CHB:	chris hani baragwanath
CMO:	cardiomyopathy
CMV:	cytomegalovirus
CPB:	cardiopulmonary bypass
CRP:	c reactive protein
CVD:	cardiovascular disease
DALY:	disability adjusted life years
D-dimer:	D-dimer
DES:	drug eluting stent
DIC:	disseminated intravascular coagulopathy
DNA :	deoxyribonucleic acid
EDTA :	ethylenediaminetetraacetic acid
E-selectin:	Endothelial selectin
FMD:	Flow mediated dialatation
HAART:	Highly active anti-retroviral therapy
HDL:	High density lipoprotein
HIV:	Human immunodeficiency virus
hs-CRP:	High sensitivity C reactive protein
ICAM-1:	Intercellular adhesion molecule 1

IL-6:	Interleukin 6
IMT:	Intima-media thickness
IRA:	Infarct related artery
IVUS:	Intravascular ultrasound
LA:	Lupus anticoagulant
LAD:	Left anterior descending coronary artery
LDL:	Low density lipoprotein
LTB:	Large thrombus burden
MACE:	Major adverse cardiovascular events
MCP-1:	Macrophage chemoattractant protein-1
MI:	Myocardial infarction
NNRTI:	Non-nucleoside reverse transcriptase inhibitor
NRTI:	Nucleoside reverse transcriptase inhibitor
NSTEMI:	Non-ST elevation myocardial infarction
PC:	Protein C
PCI:	Percutaneous coronary intervention
PI:	Protease inhibitor
PS:	Protein S
STEMI:	ST-elevation myocardial infarction
TM:	Thrombomodulin
TIMI:	Thrombolysis in myocardial infarction
TNF-α:	Tumour necrosis factor α
TLR:	Target lesion revascularization
UA:	Unstable angina

VCAM-1:	Vascular cellular adhesion molecule 1
vWF:	Von Willebrand factor

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CHAPTER ONE: INTRODUCTION AND BACKGROUND TO THE THESIS

1.1 INTRODUCTION TO THE THESIS

South Africa is thought to be a country in epidemiologic transition facing an epidemic of cardiovascular disease (1). The spectrum of heart disease facing the country ranges from the so-called traditional forms of infectious diseases to newer non-communicable diseases, with a high prevalence of modifiable risk factors that are often reported in high income countries (2, 3). Rates of cardiovascular disease in the predominantly black population of Soweto ,which have traditionally been low, are on the increase (4); this "double burden" of disease is particularly concerning, considering the current human immunodeficiency virus (HIV) pandemic, with an estimated: 5.5 million South Africans infected, one million having AIDS and five hundred thousand HIV-related deaths annually (5).

While cardiac complications of HIV infection are well described (6), controversy still exists as to whether HIV seropositive subjects have a higher incidence of coronary artery disease (CAD) and acute coronary syndromes (ACS). Autopsy studies in HIV positive patients from the developed world have shown unexpectedly high rates of atherosclerotic CAD compared to aged-matched HIV negative patients (7).

The phenomenon of premature CAD and related events in HIV positive patients can be directly attributed to the virus and/or treatment related factors. HIV infection itself may

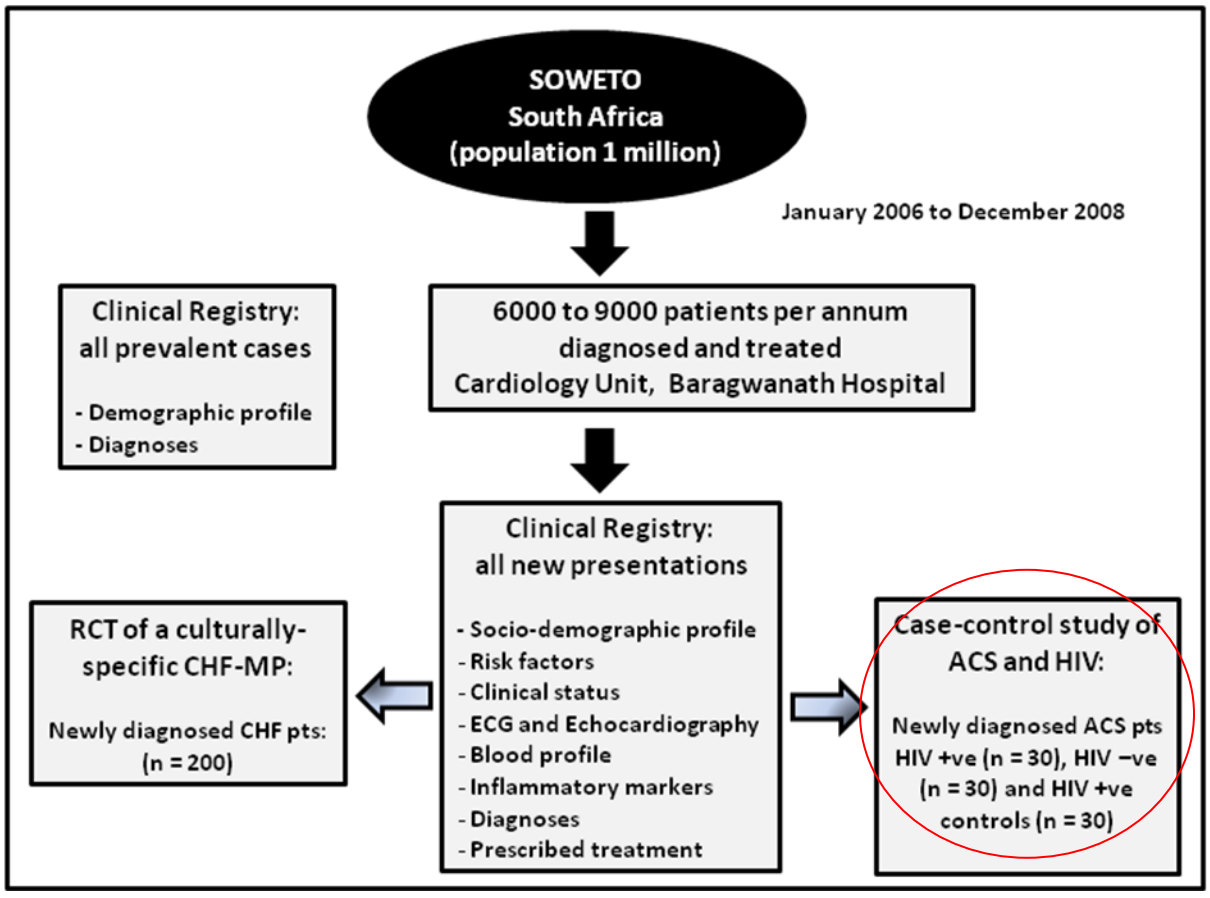
independently predispose to premature atherosclerosis through endothelial dysfunction (8), a heightened proinflammatory state (9, 10) and dyslipidaemia characterized by reductions in high density lipoprotein (HDL) cholesterol and elevations in triglycerides (11, 12). HIV patients are also at higher risk for thrombosis, owing to various abnormalities of the coagulation and fibrinolytic systems, resulting in a prothrombotic state (13-15). Alternatively, protease inhibitors (key drugs in certain anti-retroviral (ARV) regimens) have the potential to induce an adverse metabolic phenotype including dyslipidemia and insulin resistance, endothelial dysfunction and a prothrombotic state (6, 14, 16).

Following an earlier report suggesting a strong link between highly active anti-retroviral therapy (HAART) and premature CAD (17), the subsequent evidence regarding the relationship between HIV, coronary artery disease and acute coronary syndromes has been conflicting (6). It seems, however, that the risk of ACS is increased by prolonged exposure to protease inhibitors (18) independent of their effect on traditional risk factors. With HAART being the standard of care for all eligible patients in affluent societies, very little data regarding CAD in treatment-naïve patients exists. This historical lack of data for treatment-naïve patients presenting with concurrent HIV and CAD has, therefore, hindered attempts to understand the fundamental role of HIV status (and by its very absence, HAART) in the development of premature CAD.

With the great majority of HIV infected patients living in Sub-Saharan Africa (19) having limited access to HAART, the lack of data is problematic, particularly in view of the

anticipated cardiovascular pandemic facing developing countries (20). The research contained in this thesis will represent a sub-study of the larger "Heart of Soweto Study" (Figure 1.1). It will systematically examine the epidemiologic transition in risk behaviours and clinical presentations of heart disease in the predominantly black African population of approximately one million people living in the townships that comprise the internationally renowned and celebrated area of Soweto.

Figure 1.1: Study schema for the "Heart of Soweto Study"



Key: RCT, Randomised Controlled Trial; CHF, Congestive heart failure; MP, Management Programme

The aim of the research contained in this thesis is to address a number of important issues regarding:

1) Epidemiologic transition ("Heart of Soweto" study, Chapter 3 and Emerging epidemic of cardiovascular disease among urban Africans: Acute coronary syndrome at Baragwanath Hospital Soweto study, chapter 4).

2) Acute coronary syndromes in the setting of HIV infection ("Heart of Soweto" sub-study, chapters 5 to 8).

The objective is to provide a better understanding and hopefully better prevention and treatment for our patients, particularly those suffering from HIV and AIDS. It is to this end that the specific research questions to be addressed by the research contained in this thesis are:

Research Question 1: (Addressed in chapters 3 and 4)

1a) What is the current spectrum of cardiovascular disease seen in a large urban population of black South Africans, including the prevalence, incidence and associated risk factors?

1b) Is this pattern consistent with epidemiologic transition?

Research Question 2: (Addressed in chapter 5)

2) Are there differences in the demographic, clinical and angiographic features as well as treatment outcomes in black South Africans presenting with ACS according to HIV status?

Research Question 3: (Addressed in chapters 6 and 7)

3) Do thrombotic profiles in black South Africans with ACS differ according to HIV status?

Research Question 4: (Addressed in chapter 8)

4) Do markers of inflammation and endothelial cell activation differ in black South Africans with ACS according to HIV status and what is their role in the pathogenesis of ACS?

1.1.1 OVERVIEW OF THE THESIS

This thesis is divided into nine chapters. The first includes this introduction to the research and a detailed literature review. The review begins with a broad epidemiological account of the current cardiovascular and HIV pandemic facing South Africa. This is followed by a more focused look at the current available literature on the cardiovascular manifestations of HIV/AIDS and the effects of anti-retroviral therapy.

The remainder of the review details the current understanding of the elements influencing coronary artery disease and acute coronary syndromes in HIV positive patients as well as the gaps in our knowledge. Chapter two discusses the methodology used to answer the research questions. Chapters three to eight present peer reviewed manuscripts, each addressing a specific research question. Chapter three presents the findings of the large epidemiological “Heart of Soweto” study (Figure 1.1), which discusses epidemiologic

transition in Soweto, South Africa. Chapter four presents the findings of an epidemiologic study done at Chris Hani Baragwanath Hospital, which looked specifically at the incidence of acute coronary syndromes as part of an emerging epidemic of cardiovascular disease in the population of Soweto. Chapter five presents the findings of a prospective case-control observational study that looked at the clinical, angiographic and outcomes data of black South African patients with HIV and ACS. Chapters six to eight report the findings of three prospective case-control observational studies and compare various parameters concerned with the possible pathogenesis of ACS in HIV positive and negative patients. Chapter six discusses thrombotic profiles; chapter seven describes the incidence and clinical correlates of antiphospholipid antibodies and the antiphospholipid syndrome; chapter eight presents data on markers of inflammation and endothelial activation. The final chapter of this thesis (chapter nine) includes an in-depth discussion, a conclusion and suggestions on the way forward for future research.

In keeping with the guidelines of the University of the Witwatersrand, each chapter in this thesis is based on published peer reviewed papers or those accepted for publication. Preceding each chapter is a signed statement of originality, required by the University, which details the contribution of each author listed on each paper. Each chapter is introduced within the context of the thesis. Permission has been granted from each journal to include a copy of the published paper in this thesis. A condition of this permission is to include the publication as it appears in the journal.

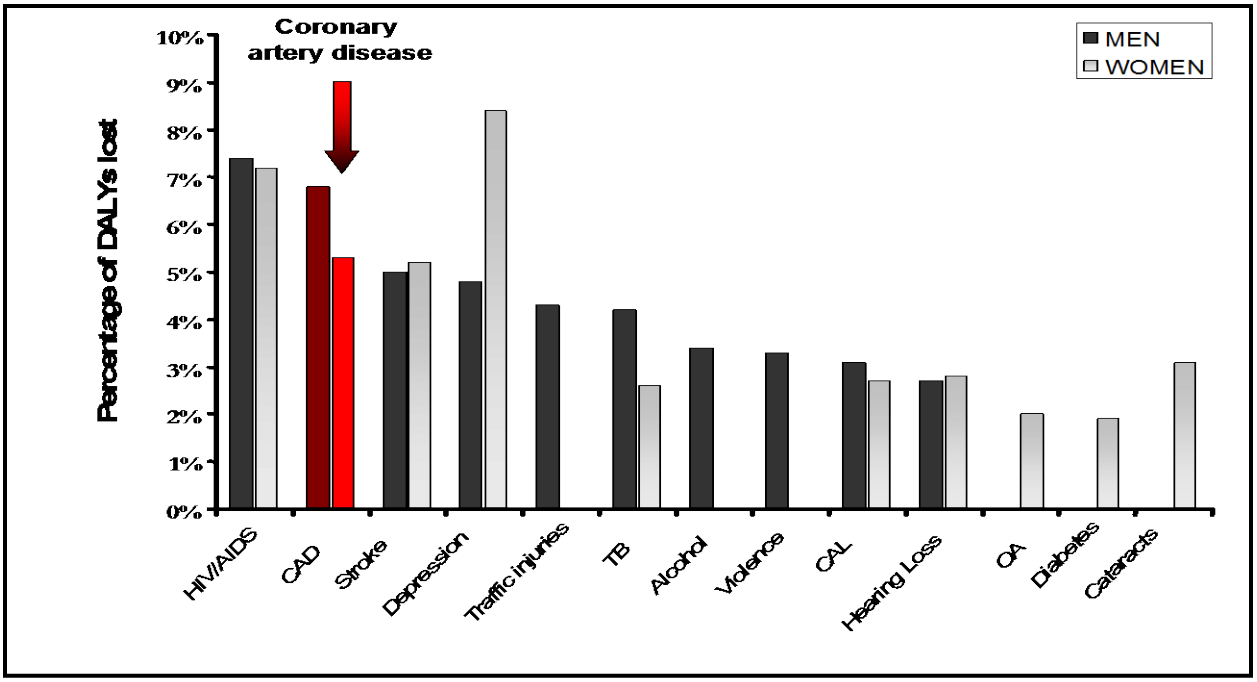
1.2 EPIDEMIOLOGY OF CARDIOVASCULAR DISEASE IN DEVELOPING COUNTRIES

Despite marked improvement in its prevention and treatment, cardiovascular disease (CVD) remains one of the largest contributors to morbidity and premature mortality in the world today. Moreover, the global burden of CVD is predicted to rise by around 50% and 150%, in the developed and developing worlds respectively, in the first quarter of the 21st Century (21) . By 2020, this burden will have increased by 130% in Africa alone, directly affecting 1.3 million people (20). Figure 1.2, specifically shows the impact of coronary artery disease (CAD), the greatest contributor to the burden of CVD overall, in terms of "healthy life-years lost" globally. Even in low to middle income countries, such as South Africa, it is responsible for close to 10% of healthy life-years lost, and is second only to HIV/AIDS in this regard.

These data support the hypothesis that the overall health status of human societies is linked to economic development. With industrialization, the major causes of death have shifted from infectious diseases and nutritional deficiencies to more chronic disorders, a phenomenon with distinct stages known as "epidemiologic transition" (21, 22). There are strong indications that in South Africa this phenomenon is occurring, even within specific disease categories, with increasing rates of CAD (23, 24) and acute coronary syndromes, (ACS) including acute myocardial infarction (AMI) (25, 26) - all common precursors of chronic heart failure. Similar to other countries in transition, South Africa is suffering a double or even triple burden from: a) the historical and lingering burden of infectious

disease; b) an emerging epidemic of chronic disease (particularly CAD); c) the re-emergence of infections such as HIV/AIDS and tuberculosis. Furthermore, as will be discussed later, HIV may in fact be adding to the burden of CAD and acute coronary events.

Figure 1.2: Impact of the most common causes of death and disability in the world population: Disability adjusted life-years lost (DALY's) (20)



Key: CAD - Coronary artery disease; TB - Tuberculosis; CAL, Congenital anomalies; OA - Osteoarthritis

This epidemiologic transition is already quite evident when considering the increasing demands for acute and outpatient cardiac clinical services from the one million people living in Soweto. For example, in a study recently published in 'The Lancet', 250 black African patients with acute myocardial infarction (AMI) were recruited as part of the INTERHEART Study (27). This large, international, standardized case control study of similar cohorts in 52 countries, examined the importance of risk factors for CAD on a

world-wide basis. During the study, 262 centers recruited men and women with a first-ever AMI and presenting to their local coronary care unit (CCU). At least one age and sex-matched control was recruited for each case of AMI. Overall 12 461 cases and 14 637 controls were analysed. On an adjusted basis, the following five "modifiable" risk factors were found to be most predictive of this event:

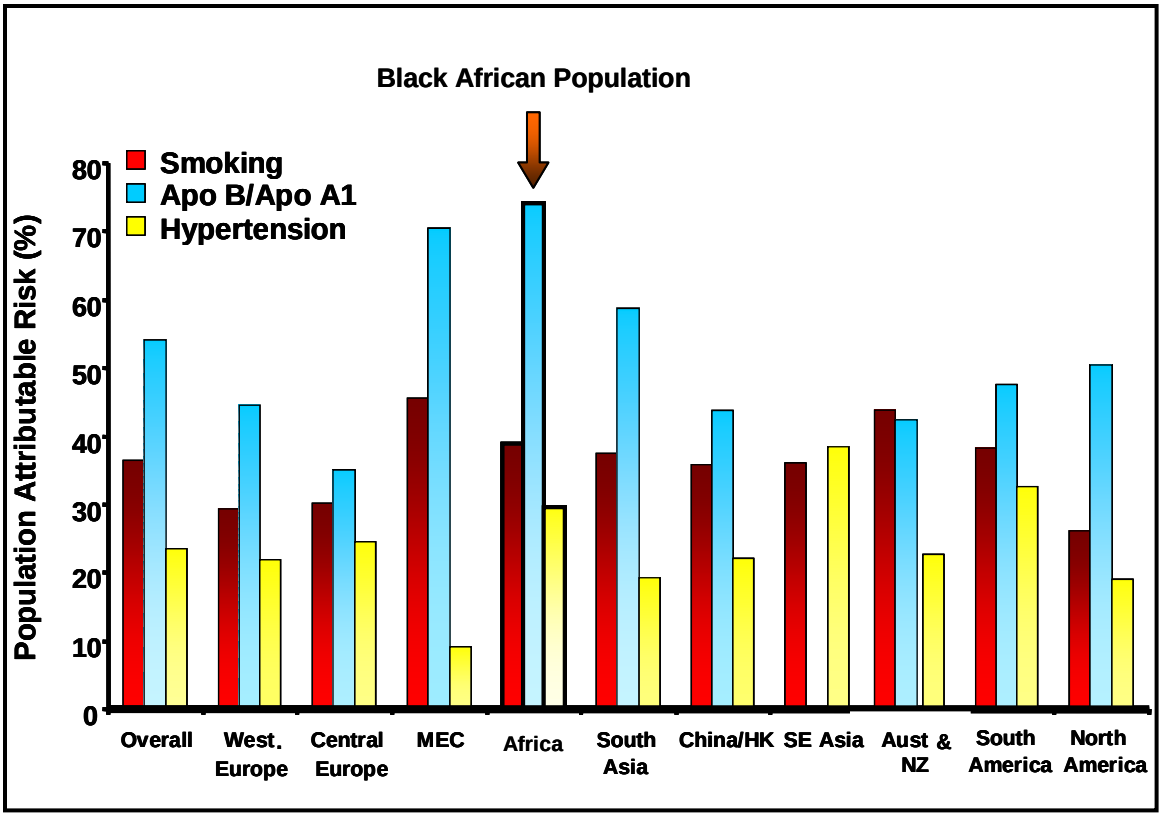
1. Current smoking: OR 2.87 (99% CI 2.58 to 3.19)
2. Raised Apoprotein B/Apoprotein A1 ratio *: OR 3.25 (99% CI 2.81 to 3.76)
3. Diabetes: OR 2.37 (99% CI 2.07 to 2.71)
4. Hypertension: OR 1.91 (99% CI 1.74 to 2.10)
5. Psychosocial Stress: OR 2.67 (99% CI 2.21 to 3.22)

** Top versus lowest quintile. Combined index comparing exposure versus non-exposure to depression, high perceived stress, poor locus of control and major life events.*

Overall, the INTERHEART Study suggests that nine risk factors amenable to modification accounted for around 90% of the risk of an incident AMI (28). Moreover, this study also demonstrated that the effect of these risk factors is consistent across a wide-range of ethnic, cultural and geographic regions across the globe: providing strong evidence that negative changes in the risk behaviour profile of the Soweto population (e.g. higher rates of obesity and hypertension) is likely to lead to a dramatic increase in the incidence of CAD. It is within this context that Figure 1.3 highlights regional differences in the population's attributable risk of AMI (reflective of the relative prevalence and risk associated with each risk factor) associated with smoking (25% to

45%), dyslipidemia (55% to 75%) and hypertension (5% to 40%), with a specific focus on the black African cohort (28).

Figure 1.3: Global comparison of the population's attributable risk of CAD for smoking, dyslipidaemia and hypertension (28)



Key: Apo B - Apoprotein B; Apo A1 - Apoprotein A1; MEC - Middle East Crescent; China/HK - China/Hong Kong; SE Asia - South East Asia; Aust & NZ -, Australia & New Zealand

Despite obvious trends in health care utilisation rates and some research reports from the African continent (26, 29), there is a paucity of data collected on a systematic basis to describe this "epidemiologic transition" in the developing world, particularly in a large vulnerable population subject to generally improved economic conditions. What is clear

is that both CVD and HIV/AIDS are significant contributors to global disability and premature mortality.

1.3 EPIDEMIOLOGY OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION IN SOUTHERN AFRICA

Southern Africa has the largest prevalence of HIV/AIDS in the world. In 2007, this sub-region accounted for almost a third (32%) of all new HIV infections and AIDS-related deaths globally, with the national adult HIV prevalence exceeding 15% in eight countries in 2005 (Botswana, Lesotho, Mozambique, Namibia, South Africa, Swaziland, Zambia and Zimbabwe) (5). Nowhere else has the national adult HIV prevalence reached such high levels. While there is some evidence of slight declines in the epidemic in some countries, notably Zimbabwe, the epidemic in most countries in the Southern African region seems to have reached or is approaching a plateau.

With an estimated 5.5 million [4.9 million-6.1 million] people living with HIV (19), South Africa is the country with the largest number of infections in the world. South Africa's Department of Health estimates that 18.3% of adults (15 - 49 years) were living with HIV in 2006 (30). More than half (55%) of all South Africans infected with HIV reside in the KwaZulu-Natal and Gauteng provinces (31). The latest data collected at antenatal clinics in South Africa suggest that infection levels may be levelling off, with HIV prevalence in pregnant women at 30% in 2005 and 29% in 2006 (30). The decrease in percentage of young pregnant women (15 - 24 years) found to be HIV positive also

suggests a possible decline in the annual number of new infections. Young women in South Africa face greater risks of becoming infected than men. Among 15-24 year-olds, women account for around 90% of new HIV infections (32). HIV incidence among 20-29 year-old women in 2005 was around 5.6%, more than six times higher than for men of the same age (0.9%) (32).

An estimated 1.8 million South Africans have died form AIDS-related disease since the epidemic began (31). The total of all cause mortality increased by 87% from 1997 to 2005 (from 316505 to 591213) (33, 34), with at least 40% of those deaths estimated to have been AIDS-related. (35). Rising death rates lowered life expectancy at birth to 49 years for males and 52.5 years for females in 2006, and has probably contributed to the decline in the country's population growth rate from 1.25% in 2001-2002 to slightly more than 1% in 2005-2006 (36).

1.3.1 SOUTH AFRICAN ANTI-RETROVIRAL ROLLOUT PROGRAMME

On 8 August 2003, the South African government made a commitment to provide anti-retroviral (ARV) treatment in the public health sector. On 19 November 2003, it published the Operational Plan on Comprehensive HIV and AIDS Care, Management and Treatment for South Africa (the Operational Plan) (37). The estimated requirement set out in the Operational Plan was for 500 000 patients to access ARV's per year. By January 2006 the total number of adult patients on ARV treatment in both the public and private sector was estimated to be around 200 000 to 220 000 - well below expectation.

About 110 000 to 120 000 people were puportedly accessing ARV's in the public sector, with an additional 90 000 to 100 000 receiving it in the private and not-for-profit sectors (38).

The lack of monitoring and evaluation of the programme has made efforts to obtain accurate statistics difficult. As in most other countries, human resources have been a major barrier to the speedy implementation of prevention and treatment programmes. In November 2005 the International Treatment Preparedness Coalition (ITPC) issued "Missing the Target: A Report on HIV/AIDS Treatment Access from the frontlines". The report detailed specific barriers and potential solutions to AIDS treatment delivery in six countries heavily affected by the pandemic, including South Africa, and made recommendations for national governments and mutilateral institutions (38). With regard to SA, the ITPC identified a lack of proper leadership, coupled with AIDS denialism as the main obstacles to increasing the number of patients on treatment. Other barriers include an acute shortage of health workers (mainly nurses and pharmacists), a lack of proper infrastructure and insufficient access to and promotion of voluntary testing (38).

The upshot of all these barriers to treatment has been that the great majority of South Africans infected with HIV have not had access to appropriate treatment, including the patients who participated in this study, who were all treatment-naïve. In June 2007, the South African Government released a guiding document with a view to comprehensively dealing with HIV from 2007-2011: the National Strategic Plan (NSP) (39). The structure of the document broadly follows four key areas, namely: 1) Prevention 2) Treatment, care

and support 3) Research, monitoring and surveillance 4) Human rights and access to justice. The primary aims of the plan are to reduce the rate of new HIV infections by 50% by 2011 and to reduce the impact of HIV and AIDS on individuals, families, communities and society by expanding access to appropriate treatment, care and support to 80% of all HIV positive people and their families by 2011 (39). The selected targets of the NSP are shown in Table 1.1.

Table 1.1: Selected targets of the National Strategic Plan

Target	**2007**	**2011**
PEP for sexual assault survivors	30% coverage	90% coverage
% of pregnant women tested for HIV	70%	95%
% of HIV-positive women given PMTCT	60%	95%
Adult population tested annually	7%	25%
New adult initiates on ART	120 000	420 000
% adult initiates started on ART outside hospital setting	30%	70%
% adult initiates started by nurses on ART	10%	80%
% HIV exposed children screened by PCR	45%	90%
New child initiates on ART	17 000	40 000
% of TB patients screened for HIV	40%	90%

PEP=Post-exposure prophylaxis, PCR=polymerase chain reaction

Considering the above mentioned ambitious aims and targets of the NSP, it is considered unlikely that these will be achieved without a radical restructuring of the current healthcare delivery system (40).

1.4 CARDIOVASCULAR MANIFESTATIONS OF HIV

1.4.1 INTRODUCTION

Although advances in highly active anti-retroviral therapy (HAART) have increased the overall survival of patients infected with HIV, the long term effects of immunodeficiency and opportunistic infections remain increasingly important (41). Heart involvement due to HIV infection was first described in 1983 by Autran et al. (42) who described a myocardial case of Kaposi's sarcoma in a patient with AIDS. Since then, the reported prevalence of cardiac involvement has ranged from 28% to 73% (43). Unfortunately most data has emanated from developed countries and little data has been reported from the developing world, where the main burden of disease lies.

The clinical expression of cardiac involvement is variable and affected by the stage of HIV disease, the degree of immunodeficiency and the use of drugs to treat HIV and/or opportunistic infections and neoplasms (44). The epidemiology of cardiovascular manifestations, like other organ systems, has changed since the introduction of HAART. On the one hand, HAART has significantly modified the course of HIV disease, lengthened survival and improved quality of life for infected patients; but on the other hand, the early data have raised concerns that HAART is associated with an increase in both peripheral and coronary artery diseases (45). The HAART-associated changes are relevant only to a minority of HIV infected individuals worldwide who have had access to the drugs and therefore, the results of studies conducted pre-HAART remain globally

applicable. Where possible, a distinction between pre and post HAART manifestations will be made. The direct effects of HAART on the cardiovascular system will be discussed in more detail in chapter 3.1 and coronary artery disease in chapter 4.

1.4.2 BIOLOGY OF HIV AND PATHOPHYSIOLOGY OF CARDIAC DISEASE

HIV was first isolated in 1983. It is a ribonucleic acid (RNA) retrovirus that uses reverse transcriptase to produce deoxyribonucelic acid (DNA) from RNA. Once in DNA form, the genetic information of HIV is incorporated as a provirus with the host cell DNA. The proviral genome can subsequently be transcribed into viral RNA that functions as messenger RNA for translation into HIV proteins and as genomes for the subsequent generation of the virus. Furthermore, HIV-1 has genes that encode the structural proteins of the virus. These are *gag* (the core of the virion, including p24 antigen), *pol* (enzymes for reverse transcription and integration), and *env* (envelope glycoprotein). Although the CD4+ T Lymphocytes and CD4+ cells of monocyte lineage are the principal targets of HIV, any cell expressing CD4 can potentially be infected with HIV. Hence cells from a variety of organs may also be affected (46).

The potential pathophysiological mechanisms of cardiac involvement by HIV are shown in Table 1.2.

Table 1.2: Potential mechanisms of cardiac disease in HIV/AIDS

MECHANISM:	EFFECT:
Direct effects of HIV:	
Infection of cardiac myocytes	Myocarditis and cardiomyopathy
Indirect effects of HIV:	
Pro-inflammatory cytokines	Impaired myocyte contractility
Anti-α-myosin autoantibodies	Endothelial dysfunction
Immune processes involving MHC-1	Cardiomyopathy
Apoptosis	
Nutritional deficiencies (carnitine,selenium,thiamine)	Cardiomyopathy
Drug toxicity: 1) Anti-retroviral treatment	
- NRTI's	Cardiomyopathy
- PI's	Dyslipidaemia, atherosclerosis, increased coronary events
2) Agents used in opportunistic infections	
- Trimethoprim/sulfamethoxazole	Prolonged QT interval,arrhythmias
- Pentamidine	Prolonged QT interval,arrhythmias
- Fluoroquinolones	Prolonged QT interval,arrhythmias
- Amphotericin B	Prolonged QT interval,arrhythmias, bradycardia, hypokalaemia
- Gancyclovir	Arrhythmias
- Foscarnet	Cardiomyopathy
- Anti-fungal agents	Prolonged QT interval,arrhythmias
3) Agents used in HIV-associated malignancies	
- Chemotherapeutic agents	Cardiomyopathy, ventricular arrhythmias, heart blocks, hypertension, CAD
- Corticosteroids	Atherosclerosis and CAD
- Interferon-α	CAD
- Interleukin-2	CAD, Cardiomyopathy

MHC= Major histocompatibility complex, NRTI= Non-nucleoside reverse transcriptase inhibitor, PI= Protease inhibitor, CAD= coronary artery disease

[Source: adapted from (47)]

1.4.3 CLINICAL SPECTRUM OF DISEASE

The clinical spectrum of cardiovascular disease in HIV/AIDS is wide and includes involvement of all three embryological layers of the heart: endocardium, myocardium and pericardium. The principle HIV-associated cardiovascular abnormalities and their reported incidence are listed in Table 1.3.

Table 1.3: The main cardiovascular complications of HIV/AIDS

CONDITION:	**INCIDENCE:**
Pericardial effusion	11% per year in asymptomatic AIDS patients before the introduction of HAART (pre-HAART)
Myocardial disease:	
Dilated cardiomyopathy	15.9 patients in 1000 asymptomatic HIV-infected persons pre-HAART
Myocarditis	1/3 of AIDS patients identified post mortem
Coronary artery disease	Incidence pre-HAART unknown Data limited to case reports post-HAART
Endocarditis	10-17% of AIDS patients identified post mortem
HIV-associated pulmonary hypertension	1/200 of HIV-infected persons pre-HAART
Systemic arterial hypertension	20-25% of HIV infected persons pre-HAART Up to 74% in HIV-infected persons with HAART-related metabolic syndrome
AIDS related tumours:	
Kaposi's sarcoma	12-28% of AIDS patients pre-HAART
Non-Hodgkins lymphoma	limited to case reports pre-HAART
Thrombosis and embolism	limited to case reports pre and post HAART
HIV vasculopathy	limited to case reports pre and post HAART
Conduction abnormalities	limited to case reports pre and post HAART

[Source: adapted from (48)]

Pericardial effusion

Pericardial effusion is the commonest manifestation of HIV infection with a reported incidence of 11% per year before the advent of HAART (49). In Africa, an estimated 70% of patients hospitalised with pericardial effusion have concomitant HIV infection with more than 90% of cases caused by tuberculosis (50).and the incidence of tuberculous pericarditis is said to be rising as a direct result of the HIV epidemic (51). The IMPI Africa (Investigation of the Management of Pericarditis in Africa) registry showed that clinically evident HIV infection had a major impact on the death rate, raising it from 17% in patients without clinical features of HIV infection to 40% in those with overt HIV disease (52). Furthermore, HIV positive patients were found to have greater evidence of myopericarditis, dyspnoea, and haemodynamic instability. These findings, if confirmed in other studies, may suggest that more intensive management is warranted in patients with HIV-associated pericardial disease (53). HIV itself can cause effusion as part of a generalised serous effusive process involving pleural and peritoneal surfaces (i.e. capillary leak syndrome related to enhanced cytokine expression in later stages) (54). The presence of effusion is an ominous sign that suggests an advanced stage of infection and is an independent predictor of mortality regardless of symptomatology (55).

Myocardial Disease

The prevalence of myocardial abnormalities among AIDS patients is 25%-75% (43). The spectrum of myocardial abnormalities includes myocarditis, dilated cardiomyopathy, ischaemic heart disease and myocardial involvement in Kaposi's sarcoma or lymphoma. Histopathologic evidence of myocarditis has been found in approximately one third of AIDS patients at autopsy, but no specific cause was identified in more than 80% of the cases (56).

Multiple pathogens have been implicated in the pathogenesis of myocarditis, including *Toxoplasma gondii*, *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, *Cytomegalovirus*, *Herpesvirus* types 1 and 2 as well as HIV itself (57). HIV-1 virions appear to infect myocardial cells in patchy distributions (58) without a clear direct association between HIV-1 and cadiac myocyte dysfunction. It is unclear how HIV-1 may enter CD4-receptor-negative cells such as myocytes. Reservoir cells (i.e. dendritic cells) may play a pathogenic role in the interaction between HIV-1 and the myocyte and in the activation of multi-functional cytokines (tumour necrosis factor-α [TNF-α], interleukin -1 [IL-1}, interleukin -6 [IL-6], interleukin -10 [IL-10]) that contribute to progressive and late tissue damage (59).

Cardiomyopathy (CMO) is now recognised as an important complication of HIV/AIDS, with an estimated annual incidence of 15.9 in 1000 before the introduction of HAART (60). The present day prevalence has been reduced by 30% in developed countries,

attributable to the introduction of HAART and the reduction of opportunistic infections (61).

The cause of CMO is complex and multifactoral including viral infections, nutritional deficiencies (selenium, carnitine, and vitamins B_1 and B_{12}), autonomic insufficiency and autoimmune factors (cytokine imbalances) (62). Increased levels of anti-α-myosin antibodies in patients with HIV and heart muscle disease have also been described (63). Compelling evidence from animal models indicates that thymidine analogues (zidovudine and stavudine) and didanosine have marked adverse effects on myocardial structure and function that are mediated by mitochondrial toxicity (64). Abnormal myocardial mitochondrial function and depletion of mitochondrial DNA have been documented in these animal models (64).

Combination ART that includes an HIV-1 protease inhibitor, by altering myocardial glucose uptake via glut-4 blockade (65), may also predispose susceptible patients to myocardial dysfunction. However, clinical data linking specific ART classes or agents with myocardial dysfunction in adult patients are lacking. CMO is usually a late manifestation of HIV disease with a poor prognosis. Various studies have shown a correlation between CMO and a poor prognosis. In patients with CMO, the median survival period is reduced to 101 days, compared with 472 days for patients who are at a similar stage of AIDS but whose hearts are normal (66).

There are presently no controlled clinical studies in the adult population to suggest the efficacy of specific therapeutic regimens for CMO in HIV and the treatment remains standard.

Endocarditis

Patients with AIDS may develop either infective endocarditis or non-bacterial thrombotic (marantic) endocarditis. Marantic endocarditis has been reported with increasing frequency in HIV-positive patients who are in the terminal stage of disease (57). The prevalence of endocarditis in autopsy studies of patients with AIDS has been reported as 10%-17%, with multi-valvular involvement being common (67). The condition is not limited to the valves and may arise or involve the endocardial lining or vascular endothelium (43). Autopsy has also revealed pulmonary and/or systemic thromboembolism in at least 40% of HIV-positive patients with thrombotic endocarditis.

The overall incidence of infective endocarditis is the same in patients with and without HIV (46) and does not seem to be affected by HAART (48). The use of intravenous drug use increases the risk for developing right-sided endocarditis. Marantic endocarditis is, however, less frequent in the post-HAART era (62). The diagnosis and management is standard.

Primary pulmonary hypertension

The incidence of primary pulmonary hypertension is estimated to occur in 0.5% of hospitalised patients with HIV (68). The pathogenesis is multifactoral and poorly understood. HIV may cause endothelial damage and mediator-related vasoconstriction through stimulation by the envelope gp120, with direct release of endothelins, interleukins and tumour necrosis factor-α (69). The development and progression of pulmonary hypertension bear no relation to the stage of underlying HIV disease (47). The prognosis for patients with this complication is poor, with reported 1-year survival rates of 51% (70). Effects of HAART regimens on the clinical course are unknown and current guidelines suggest similar treatment as for patients without HIV.

Systemic hypertension

The prevalence of systemic hypertension in HAART-naïve HIV positive black South Africans is lower than the general population (71). In a large population-based survey of body mass index (BMI) and blood pressure (BP) in rural KwaZulu-Natal, South Africa, an area of high HIV prevalence, it was shown that HAART-naïve HIV positive patients had significantly lower BMI's and systolic blood pressure (SBP) compared to age and sex matched HIV negative individuals. Furthermore, after adjusting for BMI and other determinants of BP, HIV remained a significant predictor for SBP possibly due to HIV-related hypoadrenalism or side effects of traditional medicines against HIV (71).

In this community in rural Africa, the epidemiologic transition from diseases of poverty to those of affluence (22), while already far advanced, has been partially halted by the advent of HIV. Obesity and hypertension- both conditions of affluence- are very common, but would be even more prevalent in the absence of untreated HIV infection (71). This data contrasts with that of the developed world where the prevalence of hypertension in HIV positive patients on treatment is significantly higher than the HIV negative population with a prevalence of up to 74% in patients with HAART-related metabolic syndrome (14). Predisposing factors include: leukocytoclastic vasculitis in small, medium and large vessels and aneurysms of the large vessels, such as carotid, femoral and abdominal aorta, with impairment to flow to renal arteries; HAART-induced insulin resistance with increased sympathetic activity; and sodium retention. Acute and chronic renal failure also contributes to hypertension in this population (72)..

Cardiac tumours

The two main types of tumours found in patients with HIV/AIDS are Kaposi's sarcoma (KS) and non-Hodgkins lymphoma (NHL). Since the introduction of HAART, the incidence of KS has decreased markedly and NHL involving the heart is infrequent (48). Cardiac KS is usually occult and rarely diagnosed during life. Pericardial involvement may rarely manifest as tamponade or constriction.The frequency of NHL in patients with AIDS is estimated to be 25 to 60 times greater than in the general population. Most NHL's affecting the heart are high grade tumours and the overall survival rate is poor (41).

Thrombosis and embolism

HIV positive patients, especially those with fat redistribution, may develop coagulation abnormalities, such as: increased levels of fibrinogen, D-dimer, plasminogen activator inhibitor-1(PAI-1) and Tissue-type Plasminogen activator antigen, or a deficiency of protein S (PS) (73, 74). These abnormalities have been associated with documented thromboses involving both veins and arteries and seem to be related to protease inhibitor-containing HAART (74). In a series of HIV positive patients with venous or arterial thrombosis, deep vein involvement most commonly in the lower extremity and secondary pulmonary emboli accounted for 66% of all thrombotic events. In this cohort, persistent antiphospholipid antibodies were the most common finding (75). An association between cigarette smoking and spontaneous thrombosis in HIV patients has been reported (76) and was reported to affect 77% of the patients in one series (75).

HIV vasculopathy

A wide range of inflammatory vascular diseases, both infective and non-infective may develop in HIV individuals. These include polyarteritis nodosa, Henoch Schönlein purpura and drug induced hypersensitivity vasculitis (45). Kawasaki-like syndrome (77) and Takayasu's arteritis (78) have also been described. Large-vessel disease may be aneurysmal or occlusive. Aneurysms may be single or multiple and may affect vessels such as the aorta or common carotid, common iliac, femoral or popliteal arteries (79).

Occlusive disease has been reported in Africa in young HIV positive patients and is less common than aneurysmal dilatation (79, 80). In both processes, the main histopathologic features are found in the adventitia, with leukocytoclastic vasculitis of the vasa vasorum and periadventitial vessels, chronic inflammation and fibrosis (80).

Conduction abnormalities

Prolonged QTc intervals and consequential torsades de pointes have been described in patients with HIV infection. In one study, up to 29% of hospitalised HIV patients were shown to have a prolonged QTc interval, without an obvious cause being apparent (81). In addition, certain drugs used commonly in patients with HIV infection may prolong the QTc interval: trimethoprim/sulfamethoxazole, fluoroquinolones, amphotericin B and pentamidine. Protease inhibitors have also been shown to block the human ether-a-go-go related gene channel and prolong QT intervals (82).

1.5 HIV AND CORONARY ARTERY DISEASE

1.5.1 INTRODUCTION

The first case report of severe premature CAD in 2 young men with HIV infection receiving protease inhibitor (PI) containing HAART was reported in 1998 (83). This was the first sign of the potentially deleterious effects of PI's on the vasculature, which has resulted in widespread research on the topic. Necropsy studies, however, had demonstrated premature CAD in HIV-infected patients even before the advent of PI's (84).

Subsequent to this, in 2000 Tabib et al. (85) described pathological findings in 15 HIV positive patients who had died aged 23-32 years, after having been seropositive for 2-5 years with minimal cardiovascular risk factors. The coronary arteries showed accelerated atherosclerosis and arteriosclerosis, with particular features intermediate between the lesions observed in common coronary atherosclerosis and atherosclerosis associated with chronic rejection of cardiac transplants, which evolves more rapidly (85).

The striking histopathological feature in this study was the presence of hyperplastic endothelial cells lining a thickened intima, characterised by the proliferation of smooth muscle cells and monocytic macrophages associated with the expression of the pro-inflammatory cytokines, TNF-α and interleukin-1α and rare lymphocytes on a framework rich in elastic fibres, resulting in endoluminal protrusions (85). The findings in this study

argued for the role of an inflammatory response to the HIV virus as a contributing aetiological factor to the development of atherosclerosis and it seems plausible that atherosclerotic lesions in HIV positive patients are of multifactoral origin.

Other forms of CAD in HIV, besides atherosclerotic disease, have been described, but are limited to case reports only. Barbaro et al.(86) described a case of HIV-associated coronary arteritis in a HAART-naïve patient, with no known cardiovascular risk factors, who suffered a fatal MI. Histologic analysis of both the anterior descending and circumflex arteries showed a dense infiltration of lymphocytes with necrosis of the intima. In-situ hybridization performed on serial sections of these arteries showed the presence of HIV-1 sequences within the arterial wall. Potential mechanisms through which HIV-1 may cause coronary arteritis are activation of pro-inflammatory cytokines and cell adhesion molecules and alteration of major-histocompatibility-complex (MHC) class 1 molecules on the surface of smooth muscle cells (87). Infected cells may also generate reactive oxygen species with the activation of factors that induce apoptosis (87). There have also been case reports on the presence of thrombophilic states associated with HIV infection causing MI. Santos et al. (88) described a case of recurrent coronary thrombosis associated with HIV infection, factor V Leiden, antithrombin deficiency and the primary antiphospholipid syndrome (APS).

Coronary artery disease in the setting of HIV seems to be a heterogenous disease having a multifactoral aetiology, including effects of the virus itself and the metabolic and immunorestorative effects of HAART. These aspects will be described in detail in section

5.2. Some controversy still exists as to whether or not the rate of coronary events is increased in HIV patients and whether or not the increase is caused solely by HAART.

The main studies addressing this question are summarised in Table 1.4.

Table 1.4: Coronary event rates in HIV patients according to protease inhibitor status

Study	Patients,n	Age,y	Follow up	Events	Findings
DAD study group (18)	23 468	39 (median)	1.6y on PI's	126 MI's	Risk of MI ↑ with increased exposure to PI combination therapy p<0.001
Bozette et al. (89)	36 766	NA	40 mo	1207 admissions for CVD	No ↑ in CVD admissions with PI's or with ↑ in duration of PI treatment
Mary-Krause et al. (90)	34 976	37.7 (mean)	33 mo	60 MI's	Risk of MI ↑in PI vs. non-PI treated patients. OR 2.56;95% CI, 1.03-6.34
Coplan et al. (91)	10 986	37 (mean)	1y	29 MI's	Risk of MI not ↑ in PI vs. non-PI treated patients. OR 1.69; 95% CI, 0.54-7.48
Holmberg et al. (92)	5672	42.6 (mean)	3.1y	21MI's	Risk of MI ↑ in PI vs non-PI treated patients. OR, 7.1; 95% CI, 1.6-44.3
Klein et al. (93)	4159	42.6 (mean)	3.6y	72 CAD events, incl 47 MI's	No difference in event rates between regimens but ↑event rates in HIV patients vs. controls
Barbaro et al. (94)	1551	35.5 (median)	36mo	25 coronary events including 13 MI's	Risk of MI ↑ in PI vs. non-PI treated patients RR, 11.5; CI, 2.7-48.5
Frankfurt HIV-cohort study (95)	4993	NA	58	29 MI's	↑MI rates in patients after the introduction of HAART

NA= not reported
CAD=coronary artery disease; CI= confidence interval; CVD= cardiovascular disease; HAART= highly active antiretroviral therapy; HIV= human immunodeficiency virus; MI= myocardial infarction; OR= odds ratio; PI= protease inhibitor
[Source: adapted from (6)]

In a large prospective observational benchmark study of 23 437 patients with HIV, conducted by Friis-Moller et al. in Denmark [D:A:D study group: Data Collection on Adverse Events of Anti-HIV Drugs (18)], which looked specifically at the long term effects of different ART regimens on coronary events, increased exposure to PI's was associated with an increased risk of MI. The relative rate of MI per year of PI exposure was 1.16 (95% confidence interval 1.10 to 1.23), whereas the relative risk per year of exposure to non-nucleoside reverse transcriptase inhibitors was 1.05 (95% confidence intervals 0.98 to 1.13). Adjustment for dyslipidaemia eliminated any CAD risk associated with non-nucleoside reverse transcriptase inhibitor use, but the association with PI's remained significant.

Considering the collective data, the suggestion is that the rate of MI is higher in patients taking protease inhibitors and that the risk increases with treatment duration.

1.5.2 CLINICAL AND ANGIOGRAPHIC FEATURES OF CORONARY ARTERY DISEASE IN HIV PATIENTS OF THE DEVELOPED WORLD

Various studies have looked at whether the clinical expression, angiographic features and management of HIV patients with CAD differs from the general population. The results of the main studies are presented in Table 1.5.

In general, HIV patients tend to be younger at first presentation of ACS compared to HIV negative patients. In this reviewed series of eight studies, the age ranged from a mean or

median of 42 to 50 years. This was 11 years younger than the HIV negative population with ACS found in the study done by Hsue et al. (96). There was a higher percentage of men compared to women (91% vs. 9%). In terms of coronary risk factors, the majority of patients (61%) were active smokers at the time of their coronary event.

Table 1.5: Clinical and angiographic features of coronary artery disease in HIV patients of the developed world

Study	Patients n	Age, y	Smoking (%)	CD4 count cells/mm^3	PI use (%)	MI on presentation n(%)	Coronary angiogram n(%)	Single vessel disease n(%)	PCI, n(%)	Stent ,n(%)	Overall restenosis n(%)	In-Stent restenosis n(%)	CABG n(%)
Hsue et al. (96)	68	50 ± 8	68	341 (3-4360)*	49	37/68(54)	56/68(82)	20/56(36)	29/68(43)	22/68(32)	15/29(52)	11/22 (50)	6/68(9)
Matetzky et al. (97)	24	47 ± 9	58	318 ± 210	71	24(100)	21/24(88)	5/21(24)	17/24(71)	17/24(71)	6/14(43)†	6/14(43)	3/24(13)
Escaut et al. (98)	17	46 ± 6	71	272 ± 185	65	11/17(65)	17/17(100)	9/17(53)	14/17(82)	11/17(65)	5/14(35)	5/11(45)	0/17(0)
Mehta et al. (99)	129††	42 ± 10	NA	313 ± 209	NA	82/106(77)	76/129(59)	26/76(35)	32/129(25)‡	NA	NA	NA	NA
Ambrose et al. (100)	51	48 ± 9	55	426 ± 290	59	34/51(67)	45/51(88)	21/45(47)	25/51(49)	21/51(41)	NA	NA	9/51(18)
Varriale et al. (101)	29	46 ± 10	55	>500 in 18/29	66	29(100)	13/29(45)	NA	10/29(35)	NA	NA	NA	3/29(10)
Boccara et al. (102)	50	43 ± 6.5	NA	456 (18-1220)*	76	49/50(98)	50/50(100)	NA	38/50(76)	38/50(76)	5/38(14)	5/38(14)	0/50(0)
Segev et al. (103)	12	48 ± 9	58	<500 in 12/12	92	2/12(17)	12	NA	12/12(100)	12/12(100)	7/12(58)	7/12(58)	0/12(0)

Data presented as mean ± standard deviation, percentages or median (range) *

N/A= not reported, PI=Protease Inhibitor, PCI= Percutaneous Coronary Intervention, CABG= Coronary Artery Bypass Graft

† 14 patients available for relook angiography; †† Patients drawn from 25 previous reports; ‡ Includes total no. revascularised by PCI or CABG.

[Source: adapted from (6)]

The proportion of patients receiving PI's ranged from 49% to 92%. Mean HDL cholesterol levels were significantly lower in HIV patients compared to HIV negative controls in the four studies in which they were reported, with levels of 0.9 ± 0.3 mmol/L (96), 0.8 ± 0.3 mmol/L (97) and 0.7 ± 0.3 mmol/L (98), with the fourth study quoting a higher percentage of HIV patients with low HDL cholesterol (88% vs. 42%, $p<0.001$) and hypertriglyceridaemia (78% vs. 46%, $p=0.001$) (102). Mean LDL cholesterol levels were lower in HIV patients compared to HIV negative controls in one study (97), but this was not found in another study (96).

In terms of angiographic features, as might be expected from a young cohort, single vessel CAD is commonly found in HIV patients and the TIMI risk score is low if an ACS is present (96). Percutaneous coronary intervention (PCI) has been used safely and effectively as a revascularisation strategy in HIV positive patients with ACS (96, 98, 100, 102, 103), with excellent angiographic success.

In terms of short term outcomes, in the eight studies reviewed (Table 1.5), only nine deaths (3.8%) occurred in hospital among 235 HIV positive patients. Several studies have raised a concern about a much higher than expected incidence of restenosis in HIV patients undergoing PCI with either angioplasty or bare metal stenting. Significantly higher rates of restenosis were shown in four of the five studies (Table 1.5) reporting data on restenosis (96-98, 103).

In the series by Hsue et al., restenosis developed in 15 of 29 HIV patients, compared with 3 of 21 non-HIV patients (52% vs. 14%, p=0.006) (96). For patients receiving stents, the restenosis rate was 11 of 22 HIV patients vs. 2 of 11 control subjects (50% vs. 18%, p=0.078). Similarly in the series by Matetzky et al (97), restenosis requiring target vessel revascularisation (TVR) occurred in 6 of 14 HIV patients, compared with 4 of 38 uninfected controls (43% vs. 11%, p=0.02)(97). The 6 HIV infected patients who underwent TVR, as compared to the 8 HIV patients who did not, had: a higher incidence of detectable HIV RNA load (5/6[83%] vs. 3/8[38%]; p=0.140 and lower CD4 count (280 ± 45 vs. 388 ± 185 x10^{-3}µL; p=0.4), although the differences were not statistically significant.

In the series by Escaut et al. (98), restenosis occurred in 43% of HIV patients treated with angioplasty alone and in 57% of patients who underwent bare metal coronary stenting (98). Segev et al (103) reported on 12 HIV patients who all received at least one bare metal stent for either an acute or chronic coronary indication. Seven (58%) patients developed angiographic restenosis, clinical restenosis or progression of CAD. All five patients with documented angiographic in-stent restenosis had a diffuse proliferative form of the disease (103). Boccara et al. (102) failed to show an increased incidence of restenosis in his series of 50 patients presenting with an ACS. Clinical restenosis, including TVR, was not statistically different between HIV positive and negative patients (14% vs. 16%, p=0.78).

The mechanism by which HIV may predispose to restenosis is thought to be related to a heightened inflammatory milieu. Patients with higher levels of inflammatory markers, such as C-reactive protein (CRP), at the time of PCI, have higher restenosis rates (104). Chronic low level inflammation in HIV patients may, therefore, contribute to their high rate of restenosis.

By inducing adhesion molecule expression on endothelial cells (105) and LDL cholesterol uptake by macrophages (106), CRP may contribute directly to atherogenesis and restenosis. No data regarding the safety and efficacy of drug eluting stents (DES) in the setting of HIV are currently available and, similarly, no data exists on the incidence of stent thrombosis.

Several patients in these studies underwent coronary artery bypass surgery (CABG), but no large series of HIV patients with long term follow up after bypass surgery have been reported (6). Initial concerns about the immunosuppressive effects of surgery and, in particular, cardiopulmonary bypass (CPB) and risk of postoperative infection seem to be unfounded (107). In a series of 37 HIV positive patients who were followed up for a mean of 28 months after cardiac surgery, 29 of whom received coronary bypass grafts, event free survival was 81% at 3 years (108). In recent years it has become apparent that the prognosis is strongly influenced by the patient's baseline condition (108). Patients with clinical AIDS, bacterial endocarditis and a history of intravenous drug abuse have a much worse outcome after cardiac surgery than patients who are simply HIV carriers (107).

1.5.3 PATHOGENESIS OF ATHEROSCLEROSIS IN HIV PATIENTS

1.5.3.1 INTRODUCTION

Atherosclerosis is characterised by a complex multifactorial pathophysiology. Inflammation in the vessel wall is now considered to play an essential role in the initiation, progression and final steps of atherosclerosis, namely destabilisation and plaque rupture (109). Classical pathologic studies show the abundant presence of inflammatory cells, like monocyte-derived macrophages and T-lymphocytes, at the site of rupture, or superficial erosions, especially at the shoulder area of the plaque cap.

These morphological characteristics are preceded by dysfunction of activated endothelial cells, which produce adhesion molecles that interact with inflammatory cells. The ability of monocyte-derived macrophages to secrete various cytokines, chemokines, growth factors and disintegrins, then leads to: activation and proliferation of smooth muscle cells, lesion progression and finally to weakening of a vulnerable plaque by matrix degradation of its fibrous cap (110). Endothelial cells play a fundamental role in the basal and dynamic regulation of the circulation and are constantly exposed to potentially noxious circulating agents, such as cholesterol, cigarette by-products and infective agents. Endothelial cells play an important role in the generation of the inflammatory response directed against such noxious stimuli.

Inflammation caused by infection has been recognised for a long time (111). In contrast, the sub-clinical inflammatory changes in the arterial wall because of cardiovascular risk factors have only recently emerged as an important pathogenic factor in atherosclerosis. Inflammatory markers such as CRP and IL-6 have strong and independent prognostic implications in patients with atherosclerotic vascular disease (109).

1.5.3.2 EFFECTS OF HIV ON ATHEROSCLEROSIS

Although HIV infection causes immunosuppression with an attenuated inflammatory response to certain opportunistic infections, HIV infection causes profound functional alterations of the endothelium resembling the sub-clinical inflammation in atheroscleosis. The mechanism of HIV-related endothelial dysfunction is not clear, but may include: lipid disorders associated with HIV infection (112), viral protein-related endothelial activation (113), effects of systemic inflammatory cytokine or chemokine dysregulation, or direct HIV infection of the endothelium and vascular smooth muscle cells (114). HIV-associated systemic inflammation may contribute to endothelial dysfunction and it has been shown that treatment of HAART-naïve HIV patients with the anti-inflammatory agent Salsalate improved endothelial dysfunction (115). HIV-associated activation of macrophages may predispose patients to endothelial dysfunction and enhance atheroma formation (12). In addition, activated endothelial and vascular smooth muscle cells can contribute to a prothrombotic milieu (116).

Progressive HIV disease is associated with accelerated T-cell proliferation, heightened T-cell activation and high levels of pro-inflammatory markers (117) that persist even after the introduction of HAART (118). The level of immune activation has been independently associated with CD4 T-cell nadir (106). Both immunodeficiency and immune reconstitution may be pro-atherogenic.

T lymphocytes play a key role in atherogenesis (119). CD4 activation promotes atherosclerosis through elaboration of pro-inflammatory cytokines, including Tumour necrosis factor-α (TNF- α) and the interleukins (120). Analogously, T-cell lymphocytes are also involved in the arteriosclerosis that develops in immune-suppressed patients after cardiac transplantation (121). Chronic low grade inflammation contributes to accelerated atherosclerosis (122). CRP levels are higher in HIV patients than in control subjects (16) and subjects with levels of this marker in the upper quartile or quintile have an elevated risk of cardiovascular events (123) . Some experimental data indicate that CRP is an active participant in the process of atherogenesis (124).

Leukocyte adherence to the endothelium is enhanced as the expression of cell adhesion molecules increase as part of the inflammatory process. Monocyte chemoattractant protein-1 (MCP-1) is a potent activator of macrophages and monocytes, stimulating them to migrate to the sub-endothelial space, where they begin phagocytosis of modified lipoproteins to become lipid-laden foam cells - an early step in atherogenesis. The data obtained from clinical and experimental studies provide support for a role of MCP-1 in the initiation and progression of atherosclerosis (125). Among HIV patients with sub-

clinical atherosclerosis by carotid and femoral ultrasound, MCP-1 plasma levels were higher and the frequency of a mutation in the promoter gene was also higher compared to HIV patients without atherosclerosis (126). Elevated circulating levels of von Willebrand factor (vWF), a glycoprotein facilitating platelet adhesion, synthesised in endothelial cells and megakaryocytes, are elevated in untreated HIV patients compared to HIV negative patients, reflecting endothelial activation. This correlates with circulating levels of inflammatory cytokines, such TNF-α , IL-6 and interferon γ (IFNγ). Plasma levels of vWF have prognostic relevance in CAD (111) and tend to decrease towards normal with HAART treatment (127).

Platelet activation is also enhanced in HIV patients (127). Cigarette smoking, rates of which are particularly high in HIV patients (6), activates platelets and increases coagulability. In addition, abnormalities of lipid metabolism, such as hypertriglyceridaemia and low levels of HDL have been described in patients infected with HIV before the advent of HAART (11, 12). Endothelial dysfunction, inflammation, platelet activation and hypercoagulability interact to enhance the atherogenic and thrombotic milieu of the arterial wall. The effects of HIV on atherosclerosis are summarised in Table 1.6.

Table 1.6: Effects of HIV on atherosclerosis

1. Endothelial dysfunction: - viral protein-related endothelial cell activation - direct infection of endothelium and vascular smooth muscle cells - effect of HIV-associated lipid disorders 2. Lipid disorders associated with HIV infection- hypertryglyceridaemia and low HDL 3. Systemic inflammatory cytokine-chemokine dysregulation 4. Enhanced atheroma formation by activated macrophages 5. Platelet activation 6. Prothrombotic state

[Source: adapted from (128)]

1.5.3.3 EFFECTS OF ANTI-RETROVIRAL THERAPY ON ATHEROSCLEROSIS

The first report of premature CAD in a patient treated with protease inhibitors (PI's) was published in 1998 (83). PI's have increasingly been linked to the occurrence of CAD by inducing dyslipidaemia, insulin resistance, increased levels of C peptide and lipodystrophy (129). Insulin resistance occurs in as much as 25% to 60% of patients treated with PI's (130). PI's lead to typical alterations in lipid metabolism commonly associated with insulin resistance i.e. decreased HDL cholesterol, increased LDL cholesterol, hypertriglyceridaemia and hyperinsulinaemia. As a consequence, there is reduced uptake of serum lipids by fat cells, increased lipolysis in the sub-cutaneous adipose tissue and increased production of lipids by hepatocytes.

Protease inhibitors are designed to target the catalytic region of two proteins that regulate lipid metabolism, namely cytoplasmic retinoic acid binding protein-1 and low density

lipoprotein-receptor-related protein (131). It has been hypothesised, although without strong experimental support, that this homology may allow PI's to interfere with these proteins and cause the metabolic and somatic alterations that develop in PI-treated patients (131). Recent data indicate that dyslipidaemia may be caused, at least in part, either by PI-mediated inhibition of proteasome activity and accumulation of the active portion of sterol regulatory element-binding-protein 1c in liver cells and adipocytes (132) or by apoprotein-CIII polymorphisms in HIV-infected patients (133).

The effects of PI's on lipid metabolism appear to be drug specific. For example: Ritonavir increases triglycerides and lowers HDL slightly, with no increase in LDL (134); whereas indinavir has no effect on lipoproteins but causes insulin resistance (135). Amprenavir has no effect on lipoproteins (134), but lopinavir/ritonavir increases triglycerides with no effect on LDL, HDL or insulin resistance (136). These studies were of short duration and involved HIV-negative subjects to isolate the effects of the drugs.

Abnormalities in body composition are frequent in HIV patients and have been reported in 20% to 30% of patients after one to two years of combination HAART (137). The type and duration of ART are strongly associated with the development and severity of lipodystrophy. Combination therapy with a PI and two NRTI's, particularly stavudine with didanosine, is most likely to induce severe lipodystrophy (112). The redistribution of body fat is manifest as fat wasting of the extremities, buttocks and face and/or central fat accumulation, including visceral adiposity, enlargement of breasts or gynaecomastia, increased neck circumference, dorsocervical fat pads (buffalo humps) and lipomas.

Although dyslipidaemia and abnormal glucose tolerance are not observed in every patient with fat redistribution, most studies describe an association between fat redistribution and a cluster of metabolic abnormalities including: insulin resistance, hypertryglyceridaemia and low serum levels of HDL (138). Untreated HIV-infected patients have endothelial dysfunction that improves with ART but that does not return to normal in the short term (139).

In contrast, one early study documented severe endothelial dysfunction in patients receiving long term, PI-based ART, but not in those receiving ART without a PI (129). Half of the patients received the older and now seldomly used PI indinavir. More contemporary studies, in which few patients received indinavir, have not confirmed a role for PI-containing ART regimens causing endothelial dysfunction (140). Studies in HIV uninfected patients show a marked effect of indinavir inducing endothelial dysfunction (141), but suggest a beneficial (142) or neutral (143) effect with the protease inhibitor combination lopinavir-ritonavir, or with atazanavir (143) on endothelial function.

In the South African context, indinavir is also no longer recommended due to problems with toxicity; atazanavir, lopinavir and saquinavir are the preferred PI's, generally used as second line ART (Appendix). The effects of PI's on the endothelium, therefore, depend on the specific agent used and there does not seem to be a class effect. Experimental models suggest that PI-induced endothelial dysfunction is mediated by reduced nitric oxide production or release, based on both clinical (144) and experimental

models (145). Specific mechanisms include reduced expression of endothelial nitric oxide synthase (145) and increased reactive oxygen species (146). Experimental data suggest that PI's may promote atherosclerosis by effects other than those on circulating lipoprotein levels or endothelial dysfunction. These include impaired cholesterol efflux from foam cells (147) and increased macrophage cholesterol ester accumulation through upregulation of the CD36 scavenger receptor (148). Impaired endothelial regrowth, due to ART after experimental arterial injury, is another potential mechanism whereby ART may predispose to cardiovascular disease (149).

PI-containing ART, therefore, seems to directly promote atherosclerosis independently of the metabolic effects described above. The mechanisms by which ART may adversely affect the vasculature are shown in Table 1.7.

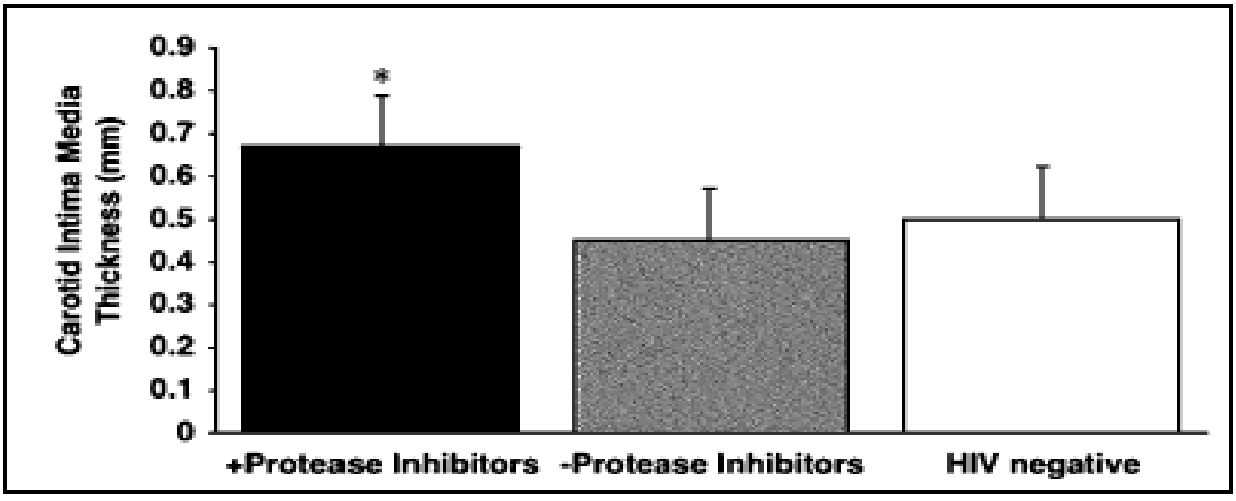
Table 1.7: Effects of ART on atherosclerosis

1. Endothelial dysfunction 2. Increased endothelial permeability 3. Increased oxidative stress 4. Increased mononuclear cell adhesion 5. Persistent arterial inflammation and immune activation 6. Accelerated lipid accumulation in the vessel wall 7. Impaired response to vascular injury 8. ART-associated lipodystrophy leading to : - metabolic syndrome - insulin resistance - mixed dyslipidaemia - hypertension - systemic inflammation - ↓ circulating adiponectin

[Source: adapted from (128)]

Carotid intima media thickness, an independent risk factor for MI and stroke (150), has been shown to be increased in patients treated with PI's compared to patients on non-PI containing regimens (151) (Figure 1.4). There is now good evidence to suggest that HIV patients on PI-containing ART regimens have a higher incidence of ACS compared to patients on non-PI containing regimens. In a prospective observational study of 23 437 patients with HIV, conducted by Friss-Moller et al. in Denmark [D:A:D study group: Data Collection on Adverse Events of Anti-HIV Drugs (18)], increased exposure to PI's was associated with an increased risk of MI. The relative rate of MI per year of PI exposure was 1.16 (95% confidence interval 1.10 to 1.23), whereas the relative risk per year of exposure to non-nucleoside reverse transcriptase inhibitors was 1.05 (95% confidence intervals 0.98 to 1.13). Adjustment for dyslipidaemia eliminated any CAD risk associated with non-nucleoside reverse transcriptase inhibitor use, but the association with PI's remained significant.

Figure 1.4: The effect of protease inhibitors on carotid intima-media thickness in HIV positive patients



Seminari et al. Atherosclerosis 2002; 162: 433-8

The pathogenesis and clinical manifestations of HIV-associated atherosclerosis may be different to common atherosclerosis. The disease seems to progress more rapidly in HIV patients, as evidenced by a specific histopathological form of the disease reported in post mortem studies (85) and evidence of rapid progression of carotid intima-media thickness (IMT) in HIV positive patients receiving PI-containing regimens compared to HIV negative controls matched for traditional risk factors (152). The reasons for this observation may be explained by the fact that HIV infection and ART act synergistically in the atherosclerotic process, which is multifactoral in HIV patients.

1.5.4 ASSESSMENT OF ENDOTHELIAL FUNCTION

The endothelium plays a key role in HIV-associated CAD in treatment-naïve patients and in those receiving HAART. The complex associations between infection, inflammation and the endothelium have long been known, but the underlying mechanisms are still not yet completely understood. Measures of endothelial function have been investigated to better understand the pathophysiology of endothelial dysfunction and (more recently) to investigate the actions of anti-retroviral drugs and estimate cardiovascular risk in HIV positive patients (153).

Endothelial function assessment can be performed either invasively or non-invasively (154). Non-invasive models include the study of biomarkers that are present on the surface of endothelial cells or are expressed in response to several stimuli and which have

an important role in the process of leukocyte rolling, firm adhesion and transendothelial migration (155). Soluble cellular adhesion molecules (CAMS) are considered reliable biomarkers of atherosclerosis development and severity and to add to the predictive value of classic risk factors for CAD in healthy individuals and in patients (156). Another non-invasive technique is the use of ultrasonography to assess the degree of flow mediated dilatation (FMD) of the brachial artery following an ischaemic stimulus, which provides a surrogate measure of the coronary circulation and a correlate of the severity of CAD (157). Another approach to studying endothelial function is to assess it invasively, by studying blood flow responses, either intracoronary or intra-arterial, to infusions of nitroglycerine and N^G-mono-methyl-L-arginine (L-NMMA) (158).

The use of non-invasive tests is clearly desirable and a recent approach to studying endothelial function in vivo in human beings has been to measure endothelial biomarkers, such as soluble CAMS, the assessment of which is non-invasive, relatively inexpensive and reproducible with low inter-operator variability.

1.5.4.1 ENDOTHELIAL CELL ADHESION MOLECULES

The adhesion to and subsequent transmigration of leucocytes across the vascular endothelium are early inflammatory events; these are mediated by endothelial CAMS and other counter-adhesive molecules present on leucocytes and other blood cells. Table 1.8 lists the main adhesion molecules and their functions.

Initial rolling of leucocytes on the vascular endothelium is mediated by members of the Selectin family (159) by creating loose contacts with the endothelial layer. P-selectin (Platelet-selectin) is stored in the Weibel-Palade bodies of endothelial cells (EC's) and is rapidly redistributed on the cell surface after agonist stimulation. E-selectin (endothelial-selectin) derives from de novo synthesis by EC's activated by inflammatory cytokines, such as IL-1 and TNF-α. E-selectin is maximally expressed 2 - 4 hours after cell activation. Within the next 24 - 48 hours, E-Selectin is again eliminated from the cytoplasmic membrane by shedding into the circulation, resulting in the circulating form or soluble (sE-selectin) (160).

Table 1.8: Endothelial cell adhesion molecules and their function

Molecules	Origin and expression	Function
Selectins:		
P-selectin	Stored in EC and platelet granules; expressed on stimulation	Loose contact of leucocytes on EC and platelets
E-selectin	Induced by cytokines on EC	Loose contact of leucocytes on EC
Immunoglobulins:		
ICAM-1	Upregulated by cytokines on EC and leucocytes	Firm adhesion and transmigration
ICAM-2	Constitutive on EC and platelets	Firm adhesion
VCAM-1	Upregulated by cytokines on EC	Firm adhesion and transmigration
PECAM-1	Constitutive on EC, platelets and leucocytes	Transmigration
TNF family:		
CD40	Constitutive on EC, leucocytes; expressed on leucocytes on stimulation	CD40Ligand is proinflammatory for EC and a platelet agonist
CD40 Ligand		

[Source: Adapted from (153)]

CD= cluster of differentiation; EC = endothelial cells; ICAM-1= intercellular adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule-1; PECAM-1= platelet endothelial cell adhesion molecule-1

Adhesion molecules involved in firm adhesion and subsequent transmigration are intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), both belonging to the immunoglobulin super-family. E-selectin is the most specific endothelial CAM, while both VCAM-1 and ICAM-1 are also produced by other cell types (lymphocytes, epithelial cells, monocytes and smooth muscle cells) (153). Since P-selectin is also contained in the platelet α-granules, from where it is released on activation, plasma P-selectin could partly indicate platelet activation (161). A significant association between increasing concentrations of soluble ICAM-1, VCAM-1 and E-

selectin and future cardiac events was shown in apparently healthy individuals in the ARIC (Atherosclerosis Risk In Communities) study (162).

1.5.4.2 HIV AND ENDOTHELIAL ADHESION MOLECULES

In the past decade, although measured by different laboratory methods, most reports agreed on increased rates of soluble adhesion molecules in HIV positive patients (153). As discussed in section 5.3.2, endothelial activation in HIV is multifactoral. The link between *in-vitro* findings and clinical data is still lacking, however. A capacity to transiently infect EC's, either directly or indirectly, would suggest that this is the main mechanism of the raised plasma concentrations.

The possible link between HIV immunological status and the concentrations of soluble CAMS as biomarkers of the severity of endothelial injury have been explored in several small cross-sectional studies. Seigneur et al. (163) conducted a study on 90 HIV-infected patients, around half of whom were taking an anti-retroviral drug (monotherapy) and half of whom were treatment-naïve. Increased levels of E-selectin (as well as thrombomodulin (TM) and vWF were seen in HIV positive individuals compared to HIV negative controls. In particular, TM was strongly raised in those with the lowest CD4 counts, whereas vWF progressively increased with each incremental drop in CD4 count, correlating with the concentrations of TNF-α and interferon-α. E-selectin; TM and vWF strongly correlated both with ß2-microglobulin (an indicator of immunological activation) and with p24 antigen (a marker of viral replication), showing that endothelial

injury was associated with progression and severity of HIV infection (163). There was also a correlation between triglycerides and VCAM-1, ICAM-1and TM levels, implying that endothelial injury may be, in part, related to certain disease-related biochemical abnormalities.

Larranaga et al. (164) looked at endothelial markers and HIV infection in the era of HAART. Endothelial markers were measured in 38 treatment-naïve patients and 63 patients receiving HAART, 33 of whom had lipodystrophy and 30 not. All endothelial markers (sVCAM-1, vWF and soluble thrombomodulin [sTM]) in the treatment-naïve group were higher compared to the patients receiving HAART. The 30 HIV patients receiving HAART with no lipodystrophy had significantly lower levels of sVCAM-1 and a trend towards lower levels of vWF and sTM. The 33 HIV patients receiving HAART with lipodystrophy had increased levels of endothelial markers compared to the HAART group with no lipodystrophy, implying that HAART decreases concentrations of endothelial activation markers but that its benefits may be offset by the metabolic syndrome that then confers a higher atherothrombotic risk (164). The use of non-invasive tools such as endothelial adhesion molecules, to assess endothelial function, although not validated, may be a useful tool in conjunction with standard risk assessments in further risk stratifying HIV patients who might benefit from more aggressive primary prevention.

1.5.5 HIV AND THROMBOSIS

Haematologic abnormalities amongst individuals infected with HIV are well described and although the cytopaenia's are the most common, there is evidence that HIV induces a hypercoagulable state, resulting in thromboembolic complications, with an incidence ranging from 0.26-7.6% (165). Autopsy studies have also revealed high rates of previously undiagnosed thromboembolism among patients with AIDS (166, 167). The mechanisms responsible for this hypercoagulable state are thought to be multifactoral in nature and involve thrombosis in both the venous and arterial beds. Thrombotic complications may arise as a direct result of the virus or indirectly through associated opportunistic infections, malignancies or the effects of HAART (14). Furthermore, it has been shown that HIV negative black Americans appear to have a greater predisposition to venous thromboembolism compared to white patients despite having less traditional risk factors for the disorder, suggesting a possible role of unrecognised heritable factors (168)

In HIV positive patients, thrombotic potential seems to correlate with the degree of immunosuppression measured by CD4 cell count as well as with the presence of opportunistic manifestations of AIDS (14). Thrombophilic states can be classified according to the vascular bed affected and the abnormality within that vascular bed, modelled on Virchow's triad (169). There may be defects within the vessel wall, defects with the blood constituents or abnormal flow i.e. stasis or combinations of all three. Table 1.9 summarises the thrombophilic state of HIV/AIDS according to this schema.

The endothelium is fundamental in the maintenance of non-thrombotic vascular surfaces and is a logical starting point when discussing thrombosis. Endothelial activation and its role in atherogenesis has been discussed in section 4.3.2, but the link between inflammation, endothelial activation and thrombosis needs elaboration.

Table 1.9: HIV and risk factors for thrombosis

Abnormality	**Affected Vascular Beds**		
	Venous	**Venous and Arterial**	**Arterial**
Defects in vessel wall:		Chronic inflammation (117),(120) Vasculitis (14)	Atherosclerosis (129) Metabolic syndrome (129) - Hypertension - Diabetes - Dyslipidaemia Smoking (170)
Defects in coagulation factors:	Deficiency of PC (171) Deficiency of PS (172) Deficiency of AT (173) Deficiency of HCII (173) ↑ Microparticles (174)		
Defects in fibrinolysis:		↑ PAI-1 (171)	
Platelet defects:		TTP (14)	↑Platelet activation (175)
Stasis:	Immobilisation (14) CCF (14)		
Other: (14)	Malignancy Opportunistic infections Nephrotic syndrome AIHA	Antiphospholipid syndrome (176) Protease inhibitors (177)	

[Source: Adapted from (169)]

PC = Protein c; PS = Protein s; AT = Antithrombin; HCII = Heparin co-factor II; PAI-1 = Plasminogen activator inhibitor-1; TTP = Thrombotic thrombocytopaenic purpura; CCF = Congestive cardiac failure; AIHA = Autoimmune haemolytic anaemia

HIV infection is characterised by a profound inflammatory response, with elevated levels of a number of pro-inflammatory cytokines, including TNF-α, IL-1, IL-6 and CRP (117, 120, 152). In addition to activating endothelial cells, these pro-inflammatory cytokines induce tissue factor expression in macrophages and endothelial cells (178). Tissue factor (TF) is a 46kD (kiloDalton) transmembrane glycoprotein that serves as a cell surface receptor and essential co-factor for coagulation factor VII and its active form VIIa (179). It is a critical factor in the initiation of coagulation in both physiologic and pathologic conditions (178). It is not normally expressed by resting endothelial cells, but can be induced by various stimuli, including vascular endothelial growth factor , TNF-α and IL-1 (180), as well as certain infectious agents.

In terms of HIV infection, there is evidence that gp120, an HIV-associated membrane glycoprotein, is able to activate human arterial smooth muscle cells *in vitro*, resulting in the elaboration of TF (181). The ability of infectious agents, immune complexes and pro-inflammatory cytokines to activate monocytes for an inflammatory response and at the same time to induce TF expression on their membrane is of great interest, as it could represent the biological basis to explain the strong relationship existing between inflammation and coagulation (178). In a recent study by Funderburg et al (182), it was shown that monocyte TF expression could be induced *in vitro* by lipopolysaccharide and flagellin , but not IL-6 . Monocyte expression of TF was correlated with HIV levels in plasma, with indices of immune activation, and with plasma levels of soluble CD14, a marker of *in vivo* lipopolysaccharide exposure. TF levels also correlated with plasma levels of D-dimers, reflective of *in vivo* clot formation and fibrinolysis. Thus, drivers of

immune activation in HIV disease, such as HIV replication, and potentially, microbial translocation, may activate clotting cascades and contribute to thrombus formation and cardiovascular morbidities in HIV infection.

Other biochemical markers of endothelial injury in HIV, besides the CAMS that have been discussed in section 5.4.2, which have important regulatory functions in haemostasis are vWF and soluble thrombomodulin (sTM). These have both been shown to be increased in HIV patients (171, 174, 183). The levels of these markers of endothelial damage correlate with the degree of immunosuppression, as measured by CD4 count. vWF is a large endothelium-derived protein with binding sites for glycoprotein Ib, glycoprotein IIb/IIIa, collagen and vitronectin, mediating platelet adhesion to other platelets and to the sub-endothelium, which is the first step in haemostasis. Thrombomodulin plays a crucial role in maintaining homeostasis of the coagulation system. It has an intracellular tail that anchors it to the endothelial cell, as well as a lectin-like domain and 6 extracellular growth factor domains (184). TM binds to and activates PC via thrombin binding regions 5 and 6 of the growth factor domains. The lectin-like domain inhibits neutrophil interaction with the endothelial cell and may, therefore, play an anti-inflammatory role. sTM is created by the proteolytic cleavage of the membrane bound molecule and increased levels indicate endothelial cell membrane injury due to neutrophil derived proteolytic enzymes and inflammatory cytokines, such as TNF-α (180).

Another triggering factor of the coagulation cascade in HIV patients could be stimulation of microparticles, i.e. small cellular remnants originating from platelets and endothelial cells found circulating in the plasma (174). Elevated levels of microparticles have been reported in HIV infected individuals (185), possibly as a reflection of CD4 lymphocyte apoptosis (186), which is associated with activation of the coagulation cascade (185). The procoagulant properties of microparticles are thought to be due to their ability to cluster coagulation proteins on the surface of activated phospholipids and act as catalysts of coagulation reactions (174).

In terms of the coagulation cascade, Antithrombin (AT) is a hepatocyte derived serine protease inhibitor that irreversibly neutralizes all the serine proteases (Factors IIa, IXa, Xa, XIa and XIIa) and is the most important physiological inhibitor of activated coagulation factors. Inhibition occurs by the formation of an equimolar complex between AT and the serine protease, a process accelerated by heparin. AT deficiency in HIV patients with thrombotic events has been described (173). The deficiency may be due to decreased hepatic synthesis from malnutrition or liver disease, protein losing enteropathy or nephropathy or consumptive states, including malignancies, disseminated intravascular coagulopathy (DIC) and surgery. Heparin cofactor 2, a specific thrombin inhibitor whose activity is enhanced by heparin, is also decreased in HIV positive individuals compared to age and sex matched healthy individuals, which correlates with the degree of immunosuppresion and confers a heightened thrombotic risk (173).

Protein C (PC) is a vitamin K-dependent protein that acts as an anti-coagulant by inactivating cofactors Va and VIIIa. Decreased levels of both functional and antigenic PC have been described in HIV patients, correlating positively with degree of immunosuppression, as measured by CD4 count (171). Factor V Leiden, which confers activated PC resistance has not been reported to date in HIV infected patients (14).

Protein S (PS) is also a vitamin K dependent plasma protein synthesized by endothelial cells, hepatocytes and megakaryocytes. It acts as a non-enzymatic co-factor of protein C (PC) in the proteolysis of activated factors V and VIII. PS circulates in 2 forms: a free active moiety (40%) and an inactive moiety that binds to C4b-binding protein (60%). PS deficiency has been reported in patients infected with HIV and is associated with a heightened thrombotic risk (172, 187). The incidence of PS deficiency seems to correlate with the degree of immunosuppression (187). The aetiology of PS deficiency is thought to be multifactorial, involving decreased synthesis and abnormal distribution between bound and free moieties. C4b binding protein is an acute phase reactant whose levels are elevated during acute inflammatory processes, reducing the active free moiety; this may partly explain the hypercoagulable state seen during acute inflammatory conditions (187).

Disorders of fibrinolysis have also been described in HIV positive patients. TNF-α has been shown to cause increased expression of PAI-1 in endothelial cells (171, 188). Coexistence of other medical conditions, such as concurrent opportunistic infections, malignancy, autoimmune haemolytic anaemia and DIC can also predispose to thrombosis (14). A review of the literature suggests that thrombosis in HIV patients is frequently

associated with the presence of opportunistic infections, particularly *Pneumocystis carinii* pneumonia (PCP) and *cytomegalovirus* (CMV) infection (15). The possible link between thrombosis and low grade DIC is supported by the fact that AIDS patients have elevated D-dimer levels and decreased levels of both antigenic and functional PC, which is thought to be due to a consumptive coagulopathy (14).

Circulating concentrations of fibrin D-dimer reflect the extent of fibrin turnover in the circulation and it has been suggested that modestly elevated circulating D-dimer values reflect minor increases in blood coagulation, thrombin formation and turnover of cross-linked intravascular fibrin, and that these increases may be relevant to coronary heart disease (189). Cigarette smoking rates have been shown to be higher in HIV infected patients compared to the general population (170) and an association between smoking and spontaneous thrombosis in HIV patients has been documented (76). Protease inhibitors (PI's) as part of highly active anti-retroviral therapy (HAART) have been associated with venous thromboembolic disease (177, 190).

It is hypothesized that PI's interfere with hepatic regulation of thrombotic proteins, which leads to a prothrombotic state in some patients (177). Platelet dysfunction has been described in HIV patients. A report by Blann et al. provided evidence for increased platelet activation, based on elevated P-selectin levels (175).

1.5.5.1 HIV AND ANTIPHOSPHOLIPID ANTIBODIES

Antiphospholipid antibodies (aPL) are a group of heterogeneous autoantibodies that have been reported in many autoimmune diseases and in the antiphospholipid syndrome (APS), which is characterised by raised levels of aPL, in association with thrombosis, recurrent foetal loss, thromboctopaenia and a number of other less commonly found complications (169). In the course of many acute infections, such as HIV, syphilis, hepatitis C, leprosy and malaria, raised levels of anticardiolipin (aCL), anti-ß$_2$-glycoprotein (anti-ß$_2$-GPI) and anti-prothrombin (aPT) antibodies have been reported. They are often transient and can disappear after treatment of the infection (191).

In HIV infection, aCL (IgG and IgM) have been reported to be present in 0-94%, anti-ß$_2$-GPI (IgG and IgM) in 4-47%, aPT (IgG and IgM) in 2-12% of patients and lupus anti-coagulant (LA) in 0-53.5%. The variations found in HIV are most likely due to the composition of the patient group and the disease stage studied, i.e. asymptomatic to full-blown AIDS and the associated presence of opportunistic infections (191). These studies were performed on predominantly white or mixed race populations.

Very little data exists on the prevalence of the IgA aPL isotype and to aPT in the setting of infections including HIV(192). Loizou et al. (192) reported on the prevalence of aPL in 100 black South African HIV positive patients. There was a low prevalence of: anti-ß$_2$-GPI (6%), all exclusively belonging to the IgA isotype; as well as aCL (7%), which were mainly positive for IgG. A prevalence of 43% (mainly IgG) aPT was found,

showing that the pattern of aPL in black South Africans differs from that found in Caucasians (192). The difference is thought to be due to infection with HIV-1 sub-types B or C, which are the viruses infecting black South Africans, as opposed to HIV-1 sub-type B, which is the predominant HIV virus found in white subjects (193).

The aetiopathogenic link between aPL and thrombosis in HIV positive patients is tenuous (191) and limited to case reports. The association of LA with thrombosis in HIV has rarely been shown (15, 176). aCL's have been associated with multiple transient ischaemic attacks and stroke (194), avascular necrosis of bone (195) and skin necrosis (196). However, in 63 HIV infected patients, Palomo et al. (197) could not find a correlation between the presence of aPL's and development of thrombosis. The clinical correlates of aPL in black South African patients with HIV are unknown. Despite this, aPL, which are often present in infectious diseases, are not usually associated with thrombotic and other complications attributed to them in patients with systemic lupus erythematosis (SLE) and APS. These infectious aPL could be induced by immune dysregulation as a consequence of the infectious process; alternatively their induction might result from the exposure of cell wall phospholipids, after breakdown on damaged body cells, as a consequence of inflammation due to the infection. The current hypothesis, therefore, is that infections (including HIV) may be a trigger for the induction of "pathogenic" aPL in predisposed or compromised subjects (192).

1.6 GAPS IN OUR KNOWLEDGE AND OBJECTIVES OF THE THESIS

Epidemiologic and disease specific data are particularly scarce in Africa. The majority of medical literature comes from the developed world, where disease patterns are different, and information regarding certain disease states cannot be extrapolated to the developing world. There is currently a paradox in that sub-Saharan Africa has the highest incidence of HIV in the world (5) and yet local data on the subject is limited, which prevents effective health care planning and ultimately health care outcomes.

HIV is a heterogeneous disease, both biologically (193) and in the way it manifests clinically, which depends on the socio-economic climate of the country, access to anti-retroviral treatment (ART) and general supportive and nutritional care. A major concern in South Africa was the view that the South African government had adopted a denialist attitude towards HIV/AIDS (38) thus impeding attempts to tackle the epidemic.

In light of this background, gaps in our knowledge need to be considered on a population based epidemiological level and on a specific disease related level. The research questions aimed at addressing the gaps in our knowledge to be addressed by this thesis are outlined below.

EPIDEMIOLOGIC TRANSITION:

While there are preliminary reports of epidemiologic transition in the developing world (198) and in South Africa particularly, there has been no formal epidemiologic study done in South Africa to document this conclusively and to provide information on the incidence and prevalence of cardiovascular disease in South Africa.

Research Question 1: (Addressed in chapters 3 and 4)

1a) What is the current spectrum of cardiovascular disease seen in a large urban population of black South Africans, including prevalence, incidence and associated risk factors?

1b) Is this pattern consistent with epidemiologic transition?

ACUTE CORONARY SYNDROMES IN THE SETTING OF HIV INFECTION

The relationship between HIV, HAART and coronary artery disease remains a highly controversial issue. Furthermore, no data exists on patients who are HAART-naïve, which makes extrapolation to the South African context inaccurate as the majority of HIV positive South Africans do not have access to HAART (38). No data currently exists on the characteristics and treatment outcomes of HIV positive black South Africans with acute coronary syndromes.

The remaining research questions will be aimed at addressing these specific issues.

Research Question 2: (Addressed in chapter 5)

2) Are their differences in the demographic, clinical and angiographic features, as well as the treatment outcomes, in black South Africans presenting with ACS, according to HIV status?

Research Question 3: (Addressed in chapters 6 and 7)

3) Do thrombotic profiles in black South Africans with ACS differ according to HIV status?

Research Question 4: (Addressed in chapter 8)

4) Do markers of inflammation and endothelial cell activation differ in black South Africans with ACS according to HIV status; and what is their role in the pathogenesis of ACS?

CHAPTER TWO: METHODS

All the studies contained in this thesis were conducted in the Department of Cardiology, CHB hospital and all adhere to the Declaration of Helsinki. Ethical clearance for all studies was granted by the Human Research Ethics Committee of the University of the Witwatersrand.

2.1 EPIDEMIOLOGICAL STUDIES

The "Heart of Soweto" (HOS) study (Chapter 3) was a prospectively designed registry that recorded epidemiologic data relating to the presentation, investigation and treatment of 1593 patients with newly diagnosed cardiovascular disease presenting to CHB hospital between January 1, 2006 and December 31, 2006. The second epidemiological study, "Emerging epidemic of cardiovascular disease among urban Africans: Acute coronary syndrome at Baragwanath hospital, Soweto" (Chapter 4), was a cross sectional study of patients with ACS admitted to the Baragwanath coronary care unit over the year 2004 compared to the years 1975-1980. For all patients presenting with ACS in 2004, we collected data on demographic factors (age, gender, ethnicity), cardiovascular risk factors, blood pressure and electrocardiogram, exercise stress test and coronary angiography. A detailed description of the methodology for these two studies is found in the corresponding publications that follow.

2.2 ACUTE CORONARY SYNDROMES IN BLACK SOUTH AFRICANS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION STUDY

2.2.1 STUDY GROUPS

Three study groups were used for analysis, as depicted below in Table 2.0:

Table 2.0: Study groups used for analysis

Group 1:	30 consecutive treatment-naïve black HIV positive patients with an Acute Coronary Syndrome **(ACS+ : HIV+ group)**
Group 2:	30 consecutive black HIV negative patients with an Acute Coronary Syndrome (**ACS+ : HIV- group)**
Group 3:	30 asymptomatic treatment-naïve black HIV positive patients without coronary disease matched for age, sex and viral load with the patients in Group 1 **(ACS- : HIV+ group)**

2.2.1.1 STUDY DESIGN AND PATIENT RECRUITMENT

The study was designed as a prospective, single centre, observational study conducted in the Department of Cardiology at CHB hospital, Soweto, South Africa. The protocol was

approved by the Human Research Ethics Committee of the University of the Witwatersrand (Protocol no. M040702) and adheres to the Declaration of Helsinki. All patients gave informed consent before study entry. Between March 2004 and February 2008, 30 consecutive black HIV patients presenting with ACS (ACS+: HIV+ group) were enrolled. For each HIV patient with ACS, we selected the first presenting black non-HIV patient with ACS as a case-control comparator (ACS+: HIV- group). In addition, a second control group, consisting of 30 asymptomatic black HIV patients matched for age and sex (ACS- : HIV+ group), were recruited from the HIV clinic.

STUDY GROUP 1 (ACUTE CORONARY SYNDROME +: HIV+ GROUP)

RECRUITMENT AND INCLUSION CRITERIA

All patients presenting with an ACS to CHB hospital during the study period were identified by our Cardiac Unit and offered HIV testing after receiving pre-test counselling. All black patients with an ACS, who were found to be HIV positive and fulfilled the inclusion criteria (Table 2.1), were recruited for the study after signing informed consent. The patients were recruited either from the medical admissions ward (ward 20) or the Coronary Care Unit (CCU) at CHB hospital.

Table 2.1: Inclusion criteria for Group1 (Acute Coronary Syndrome +: HIV+ group)

1. Informed consent
2. Age ≥18 years
3. Black ethnicity
4. HIV seropositive (Screening ELISA + confirmatory Western Blot)
5. Confirmed ACS (Unstable Angina/Non-ST elevation Myocardial Infarction/ ST-elevation Myocardial Infarction)
6. No contra-indications for coronary angiography

STUDY GROUP 2 (ACUTE CORONARY SYNDROME +: HIV- GROUP)

RECRUITMENT AND INCLUSION CRITERIA

For each patient in the ACS+:HIV+ (Group1) group recruited, the next available HIV negative black patient, with an ACS that fulfilled the inclusion criteria (Table 2.2), was recruited for this group. In terms of group 1, this group was also recruited either from the medical admissions ward (ward 20) or the Coronary Care Unit (CCU) at CHB hospital.

Table 2.2: Inclusion criteria for Group 2 (Acute Coronary Syndrome +: HIV- group)

1. Informed consent
2. Age ≥ 18 years
3. Black ethnicity
4. HIV seronegative (Screening ELISA + confirmatory Western Blot)
5. Confirmed ACS (Unstable Angina/Non-ST elevation Myocardial Infarction/ ST-elevation Myocardial Infarction)
6. No contra-indications for coronary angiography

DEFINITION OF ACUTE CORONARY SYNDROME

Acute Coronary Syndrome was defined as either ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI) or unstable angina (UA). Myocardial infarction (MI) was diagnosed in patients with compatible symptoms with elevated troponin T measurements. Patients with persistent ST elevation >0.1 mV in 2 contiguous leads were categorized as having STEMI and those without this criterion as having NSTEMI. Unstable angina was diagnosed in patients hospitalized with worsening angina or new-onset angina at rest without troponin T elevations.

STUDY GROUP 3 (ACUTE CORONARY SYNDROME -: HIV+ GROUP)

RECRUITMENT AND INCLUSION CRITERIA

The patients in group 3 (ACS-: HIV+) were selected as a second control for group 1, matched with respect to age, sex and ethnicity. The patients were screened and selected from the HIV Clinic at CHB hospital. For each patient in group 1, a patient in group 3 was selected if the patient met the criteria stipulated in Table 2.3 and if the patient was of the same sex and age (to ± 5 years) of the index patient in group 1. All patients in this group had a detailed history and examination, including resting and effort electrocardiograms (where indicated), which were used to exclude underlying coronary artery disease.

Table 2.3: Inclusion criteria for Group 3 (Acute Coronary Syndrome -: HIV+ group)

1. Informed consent 2. Age> 18 years 3. Black ethnicity 4. HIV seropositive (Screening ELISA + confirmatory Western Blot) 5. No evidence of coronary artery disease 6. No history of thrombotic events 7. No evidence of acute infectious disease 8. Not receiving antiretroviral therapy

2.2.1.2 CLINICAL DATA COLLECTION

Clinical data was obtained in all patients by taking a detailed history, clinical examination and accessing available clinical records. Patients were categorized as having diabetes, hypertension or dyslipidemia when being treated chronically for these conditions or when diagnosed with the condition on admission. Patients were classified as having "other" coronary risk factors if any of the following conditions were present: i) Family history of premature CAD (men <55 yrs, women <65 yrs); ii) chronic kidney disease; iii) post menopausal state, and; iv) abdominal obesity (abdominal circumference > 102cm in men and 88cm in women) . "Multiple" risk factors was defined as ≥ 2 of: hypertension, diabetes mellitus, hyperlipidaemia, smoking or family history.

The TIMI risk score for STEMI and NSTEMI / UA was calculated in all patients with an ACS as a clinical assessment of risk and prognosis. Demographic data was recorded for each patient and anthropometric measurements, including weight, height, body mass index (BMI), waist to hip ratio and abdominal circumference (AC) were measured on

admission. HIV positive patients were staged according to the CDC staging system, which uses two parameters, i.e.:

- Clinical category [A: Asymptomatic HIV infection; B: Non-AIDS defining symptomatic conditions; C: AIDS defining conditions]
- CD4 count (cells/ml^3) [1: ≥500; 2: 200-499; 3: <200].

2.2.2 CLINICAL BLOOD TESTS

Infection with HIV was diagnosed with the standard enzyme linked immunosorbent assay (ELISA) and Western blot techniques, after obtaining consent and offering pre-test counselling. In the HIV group, Plasma HIV RNA level was determined by quantitative polymerase chain reaction. CD4 count was determined by flow cytometry. In addition, all patients had venous blood drawn (in the fasting state) for glucose and a lipogram.

2.2.3 CORONARY ANGIOGRAPHY AND PERCUTANEOUS CORONARY INTERVENTION (PCI)

All patients in groups 1 and 2 (ACS group) were managed in the Coronary Care Unit at CHB hospital by the attending cardiology staff according to standard ACS guidelines recommended by the European Society of Cardiology. All patients underwent coronary angiography at some stage during their acute stay in the unit, the timing being left to the judgement of the attending cardiology staff.

Coronary angiography and PCI were performed in the CHB cardiac catheterisation laboratory, utilising a Philips Allura, single plane system (Koninklijke Philips Electronics, Netherlands) with standard catheters. Intravascular ultrasound (IVUS) was performed in patients found to have normal appearing infarct related arteries (IRA) at the time of angiography and in patients with evidence of in-stent restenosis at the time of follow up angiography.

All IVUS studies were performed with a commercially available system (Clearview Ultra, Boston Scientific, Boston, Massachusetts). A 40 MHz IVUS catheter was advanced distal to the culprit lesion and imaging performed in a retrograde fashion to the aorto-ostial junction. The IVUS images were recorded on VHS videotape for off-line analysis. The results of all diagnostic angiograms and PCI were assessed by two independent interventional cardiologists blinded to the patients' HIV status. Angiographic follow-up was planned for all patients receiving a coronary stent at a minimum of six months post-procedure to assess for the development of in-stent restenosis.

Single vessel disease was defined as a single major epicardial coronary artery with a stenosis ≥50% and multi-vessel disease ≥2 major epicardial arteries with stenoses ≥50%. The IRA was defined by reviewing each patient's angiogram, electrocardiogram and/or echocardiogram. An artery was considered to be infarct related if an obvious thrombus or ruptured plaque was present, or if two of the aforementioned diagnostic tests implicated the same coronary territory. The IRA was defined as angiographically normal if the

contour was smooth with no angiographic features of atherosclerotic disease in any of the coronary arteries. IRA thrombus burden, when present, was classified angiographically as small or large, according to a previously published descriptive model with 5 grades (Table 2.4).

Table 2.4: Angiographic classification of intracoronary thrombus burden

- Grade 0: No cineangiographic characteristics of thrombus
- Grade 1: Possible thrombus present
- Grade 2: Definite thrombus, with greatest dimensions ≤ ½ the vessel diameter
- Grade 3: Definite thrombus, but with greatest linear dimension >½, but <2 vessel diameters
- Grade 4: Definite thrombus, with the largest dimension ≥ 2 vessel diameters
- Grade 5: Total occlusion (unable to assess thrombus burden due to total vessel occlusion). Reclassified after flow established with guide wire or small deflated balloon passage

Small thrombus burden (STB) <Grade 4

Gibson et al. Circulation 2001; 103:2550-2554

Initial flow in the IRA was defined by the TIMI flow grade scoring system, which is a widely adopted scoring system (199): 0-3 referring to levels of coronary blood flow. TIMI 0 flow (no perfusion) refers to the absence of any antegrade flow beyond a coronary occlusion. TIMI 1 flow (penetration without perfusion) is faint antegrade coronary flow beyond the occlusion, with incomplete filling of the distal coronary bed. TIMI 2 flow (partial reperfusion) is delayed or sluggish antegrade flow with complete filling of the distal territory. TIMI 3 flow (complete perfusion) is normal flow that fills the distal coronary bed completely.

Successful PCI was defined as achievement of normal (TIMI 3) coronary flow with a residual stenosis of ≤ 50% and no periprocedural complications. Stent thrombosis was defined according to the Academic Research Consortium (ARC) criteria (200), which considers the *timing* and *probability* of occurrence of stent thrombosis. With respect to *timing*, stent thrombosis was defined as: *acute* if it occured between 0 and 24 hours; *sub-acute* when occurring between 25 hours and 30 days; *late* when occurring between 31 days and 1 year, and; *very late* when occurring beyond 1 year after stent implantation.

With respect to *probability*, stent thrombosis was defined as *definite, probable or possible*. *Definite* stent thrombosis was considered to have occurred on the basis of either angiographic or pathological evidence. *Probable* stent thrombosis was considered to have occurred in the case of: 1) unexplained death within the first 30 days after stent implantation, or; 2) MI in the territory of the implanted stent, in the absence of another obvious cause, without angiographic confirmation of stent thrombosis and regardless of its timing after the index procedure. *Possible* stent thrombosis was considered to have occurred as a cause of any unexplained death past 30 days after stent implantation.

Binary angiographic in-stent restenosis was defined as a >50% diameter stenosis at follow up. In-stent restenosis was further defined as focal or diffuse according to a previously proposed classification (Table 2.5, Methods Appendix). Target lesion revascularization (TLR) was defined as any repeat revascularization of the IRA involving the stent and/or its 5mm proximal or distal edges. Major adverse cardiovascular events

(MACE) were defined as death, non-fatal myocardial infarction or TLR. All patients were followed up at the CHB cardiac clinic.

Table 2.5: Classification of in-stent restenosis (ISR)

- Class 1: Focal ISR group. Lesions are ≤ 10mm in length and are positioned at the unscaffolded segment (i.e. articulation or gap), the body of the stent, the proximal or distal margin (but not both), or a combination of these sites (multifocal ISR).
- Class 2: "Diffuse intrastent" ISR. Lesions are >10mm in length and are confined to the stent(s), without extending outside the margins of the stent(s).
- Class 3: "Diffuse proliferative" ISR. Lesions are >10 mm in length and extend beyond the margins(s) of the stent(s).
- Class 4: ISR with "total occlusion". Lesions have a TIMI flow grade of 0.

Mehran et al. Circulation 1999; 100:1872-1878

2.2.4 RESEARCH BLOOD TESTS

The list and timing schedule of research blood tests performed for each group is listed in Table 2.6.

Table 2.6: Research blood schedule according to study group

	(TIMING ACCORDING TO GROUP[†])		
RESEARCH BLOOD TESTS	**BASELINE**	**6 WEEKS**	**6 MONTHS**
1) Markers of inflammation and endothelial activation			
Tumour necrosis factor-α (TNF-α)	1, 2, 3		1, 2
Interleukin-6 (IL-6)	1, 2, 3		1, 2
High sensitivity C-reactive protein (hs-CRP)	1, 2, 3		1, 2
Intercellular adhesion molecule-1 (ICAM-1)	1, 2, 3		1, 2
Vascular cellular adhesion molecule-1 (VCAM-1)	1, 2, 3		1, 2
Endothelial-selectin (E-selectin)	1, 2, 3		1, 2
Macrophage chemoattractant protein-1 (MCP-1)	1, 2, 3		1, 2
2) Coagulation assays			
Protein C	3	1, 2	
Protein S	3	1, 2	
Fibrinogen	3	1, 2	
Antithrombin	3	1, 2	
Factor VIII	3	1, 2	
Activated protein C resistance	3	1, 2	
D Dimer	3	1, 2	
von Willebrand factor antigen	3	1, 2	
von Willebrand factor activity	3	1, 2	
Lupus anti-coagulant	3	1, 2	
Platelet aggregometry	3	1, 2	
3) Antiphospholipid antibodies (aPL)			
Anticardiolipin (IgG)	1, 2, 3		1, 2
Anticardiolipin (IgM)	1, 2, 3		1, 2
Anticardiolipin (IgA)	1, 2, 3		1, 2
Beta-2 Glycoprotein (IgG)	1, 2, 3		1, 2
Beta-2 Glycoprotein (IgM)	1, 2, 3		1, 2
Beta-2 Glycoprotein (IgA)	1, 2, 3		1, 2
Anti-Prothrombin (IGG)	1, 2, 3		1, 2
Anti-Prothrombin (IGM)	1, 2, 3		1, 2
Anti-Prothrombin (IGA)	1, 2, 3		1, 2

[†]Group 1 = ACS+: HIV+ group; Group 2 = ACS+: HIV- group; Group 3 = ACS-: HIV+ group

2.2.4.1 BLOOD COLLECTION

For all coagulation assays and platelet studies, which were performed within 1 hour of collection, twenty ml of venous blood was obtained by clean venipuncture and drawn into plastic tubes containing sodium citrate in a ratio of 1 volume trisodium citrate to 9 volumes of blood. The samples were transported to the coagulation laboratory of the National Health Services Laboratory (NHLS), Johannesburg hospital, at ambient temperature. The samples were then centrifuged at 3500 rpm for 15 minutes prior to analysis.

For the markers of inflammation and endothelial activation, as well as antiphospholipid antibody analysis, blood was drawn, centrifuged and frozen for analysis at a later stage. Twenty ml of blood was obtained by clean venipuncture, with seated subjects and non-fasting venous blood drawn into plain tubes (for the evaluation of antiphospholipid antibodies and hs-CRP) and plastic tubes [containing ethylenediaminetetraacetic acid (EDTA)] to obtain plasma for the inflammatory cytokine and endothelial activation marker analysis. Plasma was separated by centrifugation at 3500 rpm for 15 minutes within 15 minutes of collection. Aliquots were stored at -70 degrees Celcius. All plasma samples used in these studies were thawed only once for analysis.

2.2.4.2 COAGULATION ASSAYS

All coagulation studies were performed in the Coagulation Laboratory of the National Health Services Laboratory (NHLS), Johannesburg hospital. The assays were performed on an Automated Coagulometer (ACL) [Instrument Laboratories, Milan, Italy], according to the standard operating procedures of the NHLS.

PROTEIN C (CHROMOGENIC ASSAY)

Principles of the assay: Protein C (PC) in plasma is activated by a specific enzyme from the Southern Copperhead snake venom. The amount of activated PC is determined by the rate of hydrolysis of a synthetic substrate S-2366, releasing a substance called p-Nitroanalin (pNA). The pNa released is measured at 405 nanometres (nm) using a spectrophotometer and this is proportional to the PC level in the range of 0-120% of normal plasma.

Calculations: Patients' values are calculated automatically by comparison with a standard curve. Reference ranges are 70-160%, as obtained from the package insert of the kit (Instrument Laboratories, Milan, Italy).

PROTEIN S (FUNCTIONAL ASSAY)

Principles of the assay: The functional activity of Protein S (PS), co-factor of PC, is proportional to the prolongation of the prothrombin time (PT) of a PS deficient plasma, to which diluted sample has been added. Activated PC (APC) is generated by activation of PS deficient plasma with Protac (1 part PS deficient plasma with 1 part Owrens buffer). Levels of activated PS in patients' plasma are measured automatically on the ACL, using bovine thromboplastin as reagent.

Calculations: The results obtained from the ACL are reported in percentage. All values are obtained from a calibration curve that is generated by the analyser for each batch. Reference ranges are 60-140%, as obtained form the package insert of the kit (Instrument Laboratories, Milan, Italy).

FIBRINOGEN (CLAUSS METHOD)

Principles of the assay: Calcium thrombin solution is added to patients' plasma; this converts fibrinogen to fibrin. The optical density of the clot is read at 470nm on a spectrophotometer. The resultant turbidity of normal fibrinogen is proportional to the fibrinogen concentration of the plasma.

Calculations: Reference range 2-4 g/l, as obtained from the package insert of the kit (Instrument Laboratories, Milan, Italy).

ANTITHROMBIN (CHROMOGENIC ASSAY)

Principles of the assay: Plasma is incubated with an excess of Factor Xa in the presence of heparin. The residual quantity of Factor Xa is determined by the rate of hydrolysis of a synthetic substrate, releasing a substance called p-Nitroanalin (pNA). The pNA release, measured at 405nm spectrophotometrically, is inveresely proportional to the antithrombin (AT) level, in the range of 0-125% of normal plasma.

Calculations: Patients' values are calculated automatically by comparison with a standard curve. The ACL calculates the activity of the tested parameters on the basis of the relative calibration curve. The patient's AT value has to be corrected on the ACL, depending on the AT value for the standard (Instrument Laboratories, Milan, Italy). The patient's' value is then multiplied by the correction factor.

ACTIVATED PROTEIN C RESISTANCE (APCR)

Principles of the assay: The kit is used for the determination of resistance to activated protein C (APC), caused by the FV: Q^{506} (Factor V Leiden mutation) in plasma from untreated individuals and from patients on oral anti-coagulant or heparin therapy. Sample plasma is incubated with the activated partial thromboplastin time (aPTT) reagent for a standard period of time. Coagulation is triggered by the addition of calcium chloride ($CaCl_2$), in the absence and presence of APC, and the time for clot formation is recorded.

Calculations: APC ratio= $\frac{\text{Clot time APC+ } CaCl_2}{\text{Clot time } CaCl_2}$

Typical Median Values: >2 Normal
: 1.7 Heterozygous ($FV:Q^{506}$)
: 1.2 Homozygous ($FV:Q^{506}$)

D- DIMER (QUANTITATIVE METHOD)

Principles of the assay: This assay utilises a D-dimer latex reagent (Instrument Laboratories, Milan, Italy), which is a suspension of polystyrene latex particles of uniform size (coated with a monoclonal antibody highly specific for the D-dimer domain) included in fibrin soluble derivatives. When a plasma containing D-dimer is mixed with latex reagent and a reaction buffer, the coated latex particles agglutinate. The degree of agglutination is proportional to the concentration of D-dimer in the sample and is determined by measuring the decrease of the transmitted light at 405nm caused by the aggregates (turbidometric immunoassay).

Calculations: Patients' values are calculated automatically by comparison with a standard curve. The ACL calculates the activity of the listed parameters on the basis of the relative calibration curve. Reference range <0.2mg/L.

VON WILLEBRAND ANTIGEN ASSAY (vWF:Ag)

Principles of the assay: The Instrument Laboratories vWF:Ag assay (Instrument Laboratories, Milan, Italy) involves a latex particle enhanced immuno-turbidometric

assay to quantify vWF:Ag in plasma. When plasma containing vWF:Ag is mixed with the latex reagent and a reaction buffer, the coated particles agglutinate. The degree of agglutination is directly proportional to the concentration of the vWF:Ag in the sample and is determined by measuring the transmitted light caused by the aggregates.

Calculations: Patients' values are calculated automatically by comparison with a standard curve. The ACL calculates the activity of the listed parameters on the basis of the relative calibration curve. Reference range: 50-150%.

VON WILLEBRAND FACTOR ACTIVITY (RISTOCETIN COFACTOR ACTIVITY)

Principles of the assay: This is a functional assay of von Willebrand factor activity that measures the ability of the antibiotic ristocetin to induce platelet agglutination in the presence of von Willebrand factor. Thus, the rate and extent of platelet agglutination is a function of the concentration and functional integrity of von Willebrand factor. Von Willebrand factor mediates platelet adhesion to subendothelial collagen following vascular injury. Ristocetin is an antibiotic which causes platelet agglutination only in the presence of large multimers of von Willebrand factor.

Calculations: The titre of the specimen is given by the dilution in which there is distinct agglutination compared to the blank. The ristocetin cofactor content (as a percentage of normal) is obtained by multiplying the titre of the specimen with the limit of detection

that is stated on the vial label. For example: if agglutination is detected in the 1:120 dilution and not the 1:160 dilution (120x0.7=84; 160x0.7=112, where 0.7 is the sensitivity of the von Willebrand reagent), the result is >84 <112%. Reference range 50-150%.

LUPUS ANTI-COAGULANTS (KAOLIN CLOTTING TIME)

Principles of the assay: Lupus anti-coagulants (LA) are naturally occuring immunoglobulins, which react against anionic phospholipids *in vitro*. LA inhibit the formation of the prothrombinase complex by interfering with the interaction between anionic phospholipids and clotting factors thereby causing prolonged times in phospholipid-dependant coagulation tests [Prothrombin time (PT), Partial thromboplastin time (PTT) and factor assays.See Methods Appendix]. Kaolin, which is a naturally occuring clay, initiates clotting through the contact factors and subsequently involves other factors in the intrinsic pathway of coagulation. The kaolin clotting time (KCT) is essentially an activated partial thromboplastin time test but without any added phospholipid. The test relies on residual cell membrane fragments and plasma lipids to provide a phospholipid surface for coagulation reactions. Kaolin is used to test for the presence of LA, as it does not contain phospholipid. In this assay, decreasing amounts of normal plasma (NP) are mixed with increasing amounts of test plasma (TP) and a KCT performed on each mixture. Clotting times are plotted against increasing concentrations of TP on arithmetic graph paper. The test is sensitive to the presence of LA and is able to identify the coexistence of a factor deficiency.

Results: The results are plotted on a graph with: time on the y axis; relative concentrations of TP to NP on the x axis. Results are reported as the time at 0.2TP:0.8NP. Plasma with KCT's above the reference range are then subjected to further confirmatory tests for LA using dilute Russell's viper venom.

LUPUS ANTI-COAGULANTS (RUSSELL'S VIPER VENOM)

Principles of the assay: Plasma with a KCT above the reference range is subjected to further testing using the following protocol to exclude the presence of LA. RVV (Russell's Viper Venom), phospholipids and calcium are added to the test plasma (LA1 screening test) to directly activate factors X and V, thereby triggering the joint cascade of the intrinsic and extrinsic coagulation pathways (Methods Appendix). Any LA contained in the sample prolongs the coagulation time as they block the phospholipids necessary for the coagulation process. If the clotting time from the LA1 screening test is above the reference range, the LA2 test is used for confirmation as it contains an excess of phospholipids, which neutralise the LA.

Results: If the LA1 screening reagent clotting time is within the normal range, no further testing is required and the presence of LA is excluded. If the LA1 screening reagent clotting time is more than 2 standard deviations (SD) longer than the mean of normal plasma, the result should be considered abnormal and investigated further with the LA2 confirmatory test..The final result is best expressed as a ratio of the clotting times of LA1

screen: LA2 confirm. LA1 screen: LA2 confirm >2 (strong LA presence); 1.5-2.0 (moderate LA presence); 1.3-1.5 (weak LA presence); <1.3 (No LA present).

2.2.4.3 PLATELET AGGREGOMETRY

Platelet aggregometry was performed in the Coagulation Laboratory of the NHLS. The assays were performed on an APACT-2 light transmission platelet aggregometer [Instrument Laboratories, Milan, Italy], according to the standard operating procedures of the NHLS.

Principle of assay: The absorbance of platelet-rich plasma decreases as platelets aggregate. The amount and rate of fall is dependent on platelet reactivity to the added agonist, if other variables (such as temperature, platelet count and mixing speed) are controlled. Aggregation responses to the agonist's arachidonic acid (1,5 mM), ADP (adenosine diphosphate, 10uM), collagen (2ug/mL) and adrenaline (10mg/mL) are measured by absorbance changes monitored on a chart recorder.

Results: Results are reported as "normal", "flat" or "reduced", depending on the degree of aggregation with each agonist: $\geq$ 50% aggregation in the face of an agonist defined as "normal", <20% aggregation defined as "flat" and >20 but <50% , defined as "reduced" .

PLATELET SENSITIVITIES

Principle of assay: Platelet sensitivities are performed on a range of decreasing concentrations of the agonist to assess for the presence of hypersensitive platelets, which aggregate at low concentrations of agonists. Full aggregation responses to <0.5mM arachidonic acid, <2µg/ml collagen and <1µg/ml ADP is defined as platelet hyperactivity.

2.2.4.4 MARKERS OF INFLAMMATION AND ENDOTHELIAL ACTIVATION

BIOPLEX CYTOKINE ASSAY

Principle of the assay (Methods Appendix): Bioplex cytokine assays are multiplex bead-based assays designed to quantitate multiple cytokines in diverse matrices, including serum samples, plasma samples and tissue culture supernatants. The system allows for the simultaneous analysis of up to 100 different cytokines in a single microplate well. The multiplexing bead technology is based on the colour coding of tiny beads, or microspheres. Each bead is dyed with a different ratio of two spectrally distinct fluorophores to create 100 distinct sets of beads. The Bioplex principle uses a bead format with the capture antigen whereas ELISA uses a plate format with the antigen in each well.

Antibody specifically directed against the cytokine of interest is covalently coupled to colour-coded 5.6µm polystyrene beads. The antibody-coupled beads are allowed to react with a sample containing an unknown amount of cytokine (test sample) or with a standard solution containing a known amount of cytokine. After performing a series of washes to remove unbound protein, a biotinylated detection antibody, specific for a different epitope on the cytokine, is added to the beads. The result is the formation of a sandwich of antibodies around the cytokine. The reaction mixture is detected by the addition of streptavidin-phycoerythrin (Streptavidin-PE), which binds to the biotinylated detection antibodies. The constituents of each well are drawn up into the flow-based Bioplex suspension array system, which identifies and quantitates each specific reaction based on bead colour and flourescence. Unknown cytokine concentrations are automatically calculated by Bioplex manager software, using a standard curve derived from a recombinant cytokine standard.

Assays performed:

By making use of this technology we were able to combine ("plex") the following related cytokines together in 3 different assays:

1) TNF-α / IL-6 (Pro-inflammatory cytokines)
2) ICAM-1 / VCAM-1 (Soluble cellular adhesion molecules)
3) MCP-1 / RANTES* (Chemokines)

} (Bio-Rad Laboratories, Hercules, CA, USA)

* Data regarding RANTES was not used in the thesis.

Details for the measurements of the above cytokines can be found in the Methods Appendix at the end of this chapter.

E-SELECTIN (Human sE-selectin immunoassay kit, Invitrogen Corporation, Carlsbad, CA)

Principle of the assay: The sE-selectin ELISA is an enzyme-linked immunosorbent assay for quantitative detection of soluble E-selectin levels in human sera. An anti-sE-selectin monoclonal coating antibody is adsorbed onto microwells. sE-selectin present in the sample or standard binds to antibodies adsorbed to the microwells; an horse radish peroxidase (HRP)-conjugated monoclonal anti-sE-selectin antibody is added and binds to sE-selectin captured by the first antibody. Following incubation, unbound enzyme conjugated anti-sE-selectin is removed during a wash step and substrate solution reactive with HRP is added to the wells. A coloured product is formed in proportion to the amount of soluble E-selectin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven sE-selectin standard dilutions, and sE-selectin sample concentration determined. Results are expressed as ng/mL.

HIGH SENSITIVITY C-REACTIVE PROTEIN ASSAY (CRP Vario, Sentinal Diagnostics, Milano, Italy)

Principle of the assay: CRP Vario is a latex immunoassay developed to accurately and reproducibly measure blood CRP levels in serum and plasma.When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody, which has been

adsorbed to latex particles, agglutination results. This agglutination is detected as an absorbance change (572nm), with the change being proportional to the quantity of CRP in the sample. Results are expressed as mg/L.

2.2.4.5 ANTIPHOSPHOLIPID ANTIBODIES

Antiphospholipid antibodies (aPL), including anticardiolipin antibodies (aCL), anti-ß$_2$-glycoprotein-I (anti- ß$_2$-GP-1) and anti-prothrombin (aPT) [IgG, IgM, IgA], were measured at baseline in all patients and at 6 months post-event in the ACS groups, using commercial enzyme linked immunosorbent assay (ELISA) kits (Orgentec Diagnostika, Mainz, Germany). The principle of the ELISA assay has been described in section 2.4.4.

The results are expressed in units, according to the manufacturer's instructions. IgG, IgM and IgA aCL are expressed as U/mL. Results for each of the three isotypes of the three types of aPL measured were considered positive when the optical density obtained for each patient exceeded that of the mean value plus 2 standard deviations (SD) of the 100 sera from black South African normal healthy subjects. The Antiphospholipid Syndrome (APS) was diagnosed in patients with ACS where the presence of aPL (aCL IgG/ IgM and/or anti- ß$_2$-GP-1 IgG/ IgM) in titres was greater than the mean plus 2SD from normal healthy subjects on two occasions, at least 12 weeks apart, as per the Sappora classification (201).

2.2.5 STATISTICAL ANALYSIS

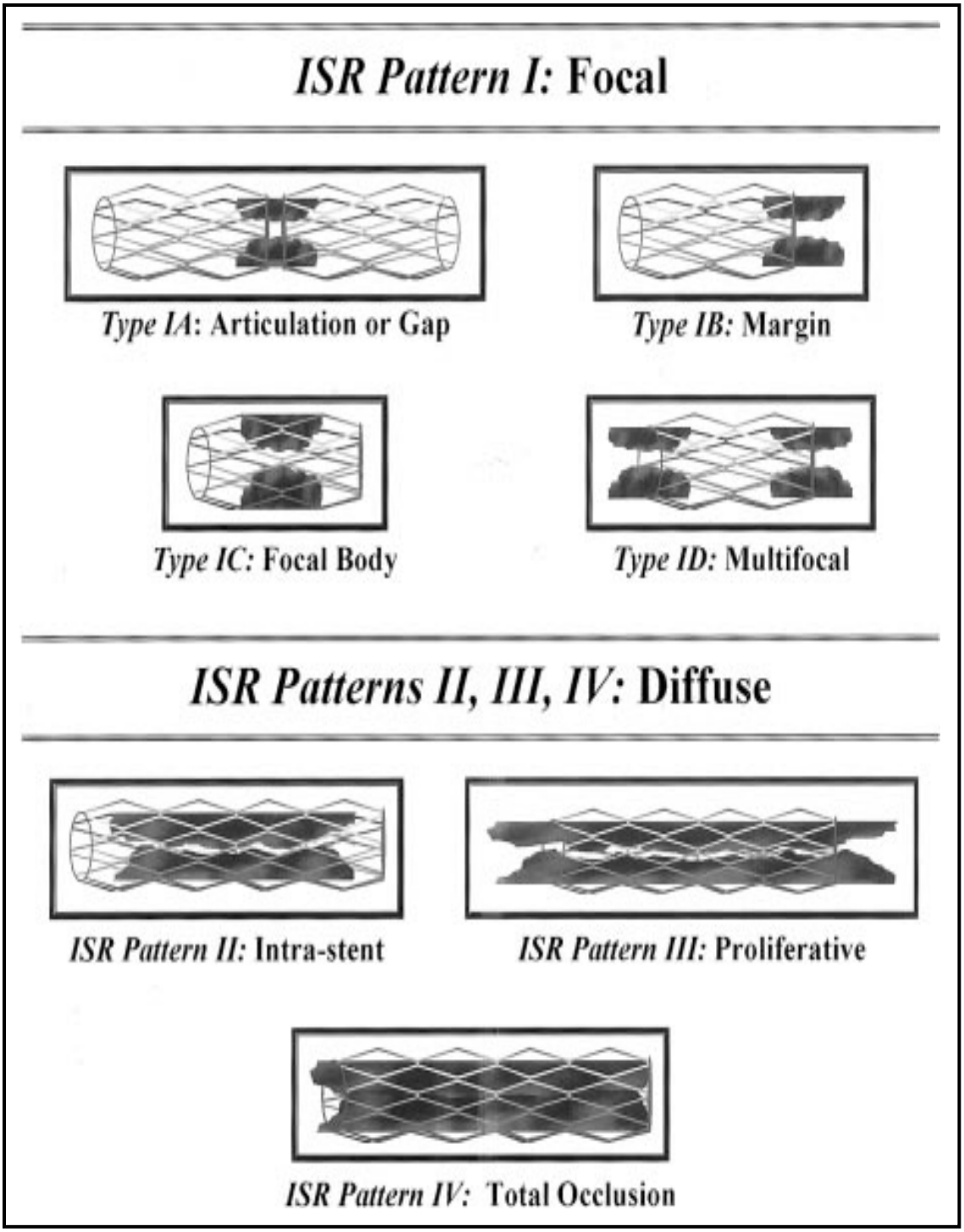
Statistical analysis was performed using SAS 9.1 software (SAS, Cary, NC, USA). Normally distributed continuous data are presented as the mean (± standard deviation), and variables, with non-Gaussian distribution as the median (min-max range). Categorical data are presented as frequencies and percentages. The initial analysis compared variables between the three groups using: the one way Anova test for continuous variables with normal distribution; and the Kruskal Wallis test in the case of non-normal distribution. For categorical variables, the Chi square test was performed with a Fisher exact test used when necessary. Significant differences between variables in the three groups was assumed at $p<0.05$. Sub-group analysis with multiple pairwise comparisons was then performed by applying the Bonferroni correction, with a p value< 0.0166 considered significant.

With specific respect to the analysis of groups 1 and 2, changes in TIMI flow rates pre and post-PCI between HIV positive and negative groups were calculated, using the Wilcoxon rank sums test. Significance was assumed at a two-tailed value of $p<0.05$. Univariate logistic regression was performed to determine predictors of the variables: normal infarct related artery, large thrombus burden and death and data presented as odds ratios (OR) with 95% confidence intervals (CI). Multivariate logistic regression was then performed, but due to the small sample size, it was not possible to include all potential explanatory variables were significantly different between the groups (age, smoking,

hypertension, total cholesterol, LDL cholesterol, HDL cholesterol and “other risk factors”); thus a limited analysis using age and smoking was performed.

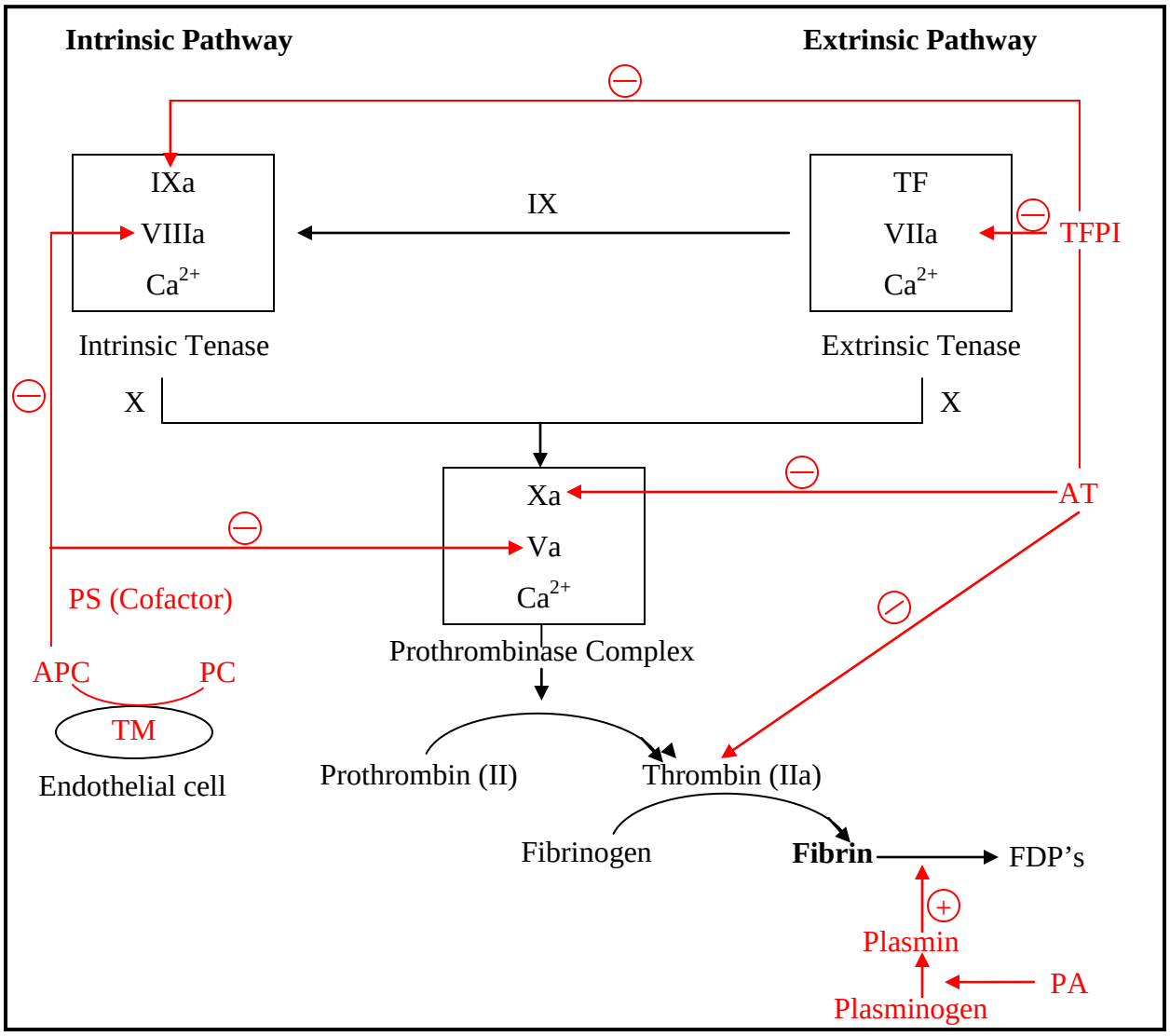
2.2.6 METHODS APPENDIX

Figure 2.1: Patterns of in-stent restenosis



Mehran et al. Circulation 1999; 100:1872-1878

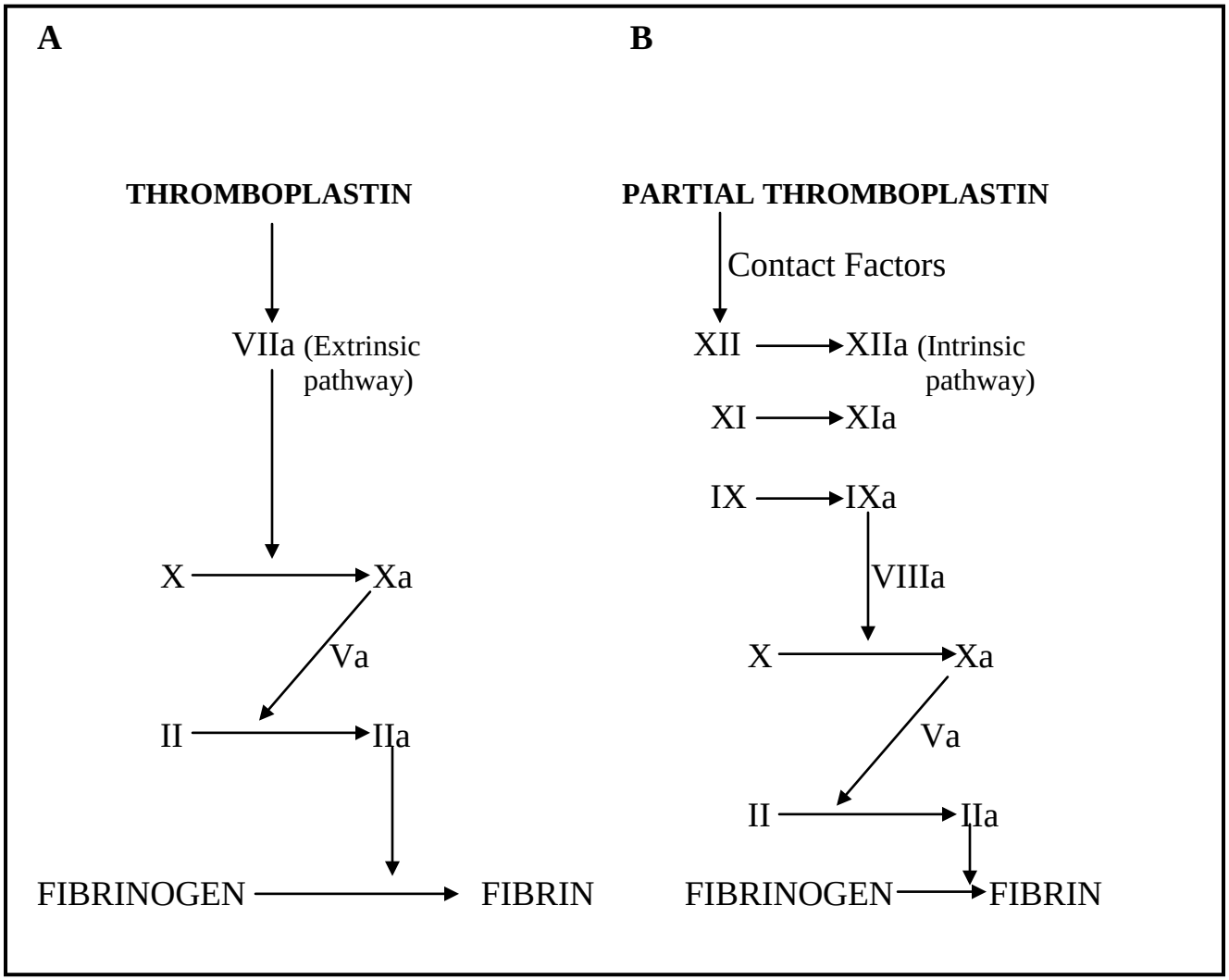
Figure 2.2: Coagulation and antithrombotic pathways



Explanation: Coagulation is initiated by extrinsic tenase, which forms when factor VIIa binds to TF. Extrinsic tenase activates factors IX and X. In the presence of calcium, factor IXa binds to negatively charged phospholipid surfaces, where it interacts with factor VIIIa to form intrinsic tenase, a complex that efficiently activates factor X. Factor Xa binds to factor Va on negatively charged phospholipid surfaces to form prothrombinase, the complex that activates prothrombin to thrombin, which converts fibrinogen to fibrin. The four main antithrombotic mechanisms and their site(s) of action are depicted in red.

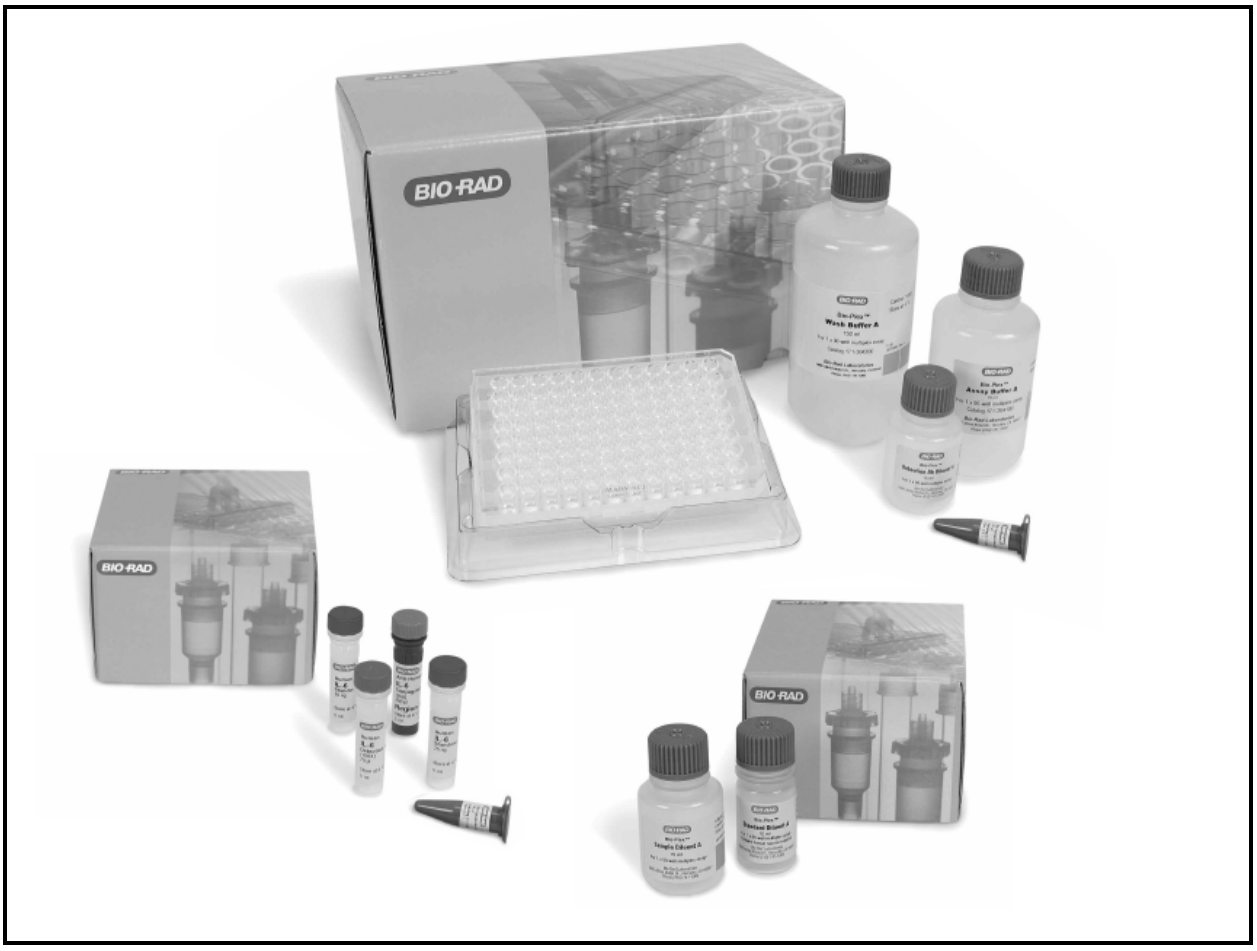
Key: APC - activated protein C; AT - antithrombin ; FDP - fibrin degradation products; PA - plasminogen activators; PC - protein C; PS - protein S; TF - tissue factor; TFPI - tissue factor pathway inhibitor; TM - thrombomodulin.

Figure 2.3: Prothrombin time (PT) and partial thromboplastin time (aPTT)



Explanation: A: **PT (Prothrombin time)**. Thromboplastin reagent containing tissue factor (TF) and calcium is added to citrated plasma. Formation of extrinsic tenase results in rapid fibrin formation via extrinsic and common pathways. B: **aPTT (activated partial thromboplastin time)**. Partial thromboplastin reagent, consisting of surface activator and dilute phospholipids, is added to citrated plasma. After incubation, to allow activation of contact factors and generation of factor IXa, calcium is added to induce clotting via intrinsic and common pathways.

Figure 2.4: Bioplex cytokine measurement



Cytokine assay kits (2-Plex) for TNF-α / IL-6, ICAM-1 / VCAM-1 and MCP-1 / RANTES (Bio-Rad Laboratories, CA, USA) were used in the analysis. The kits included beaded 96 well plates**, s**tandard and serum diluent, wash buffer, cytokine standard, detection antibody and Streptavidin-PE solution.

1) Patient plasma samples, which had been stored at -70 degrees Celcius, were allowed to thaw overnight, but were kept at 4 degrees Celcius until the first incubation.

2) The cytokine standard samples and serum were serially diluted four-fold with the use of diluent into sterile 1.5ml microcentrifuge tubes, using multi-channel micropipettes.

3) The standard and serum samples were then added to the 96 well plates and incubated for 30 minutes at room temperature.

4) The samples were then washed three times with 100µL of Bioplex wash buffer. Excess buffer was removed by vacuum filtration after each wash cycle.

5) The plate was stored in a dark environment, while the detection antibody vial was centrifuged prior to pipetting to collect the entire volume at the bottom of the vial.

6) The detection antibody was then diluted to a 1x concentration using detection antibody diluent added to the wells. Following this, the samples were incubated at room temperature for 30 minutes and washed in a similar fashion.

7) The samples were then washed again (three times) with 100µL of Bioplex wash buffer. Excess buffer was removed by vacuum filtration after each wash cycle.

8) Streptavidin-PE was then diluted to a 1x concentration with Bioplex assay buffer and added to the wells, incubated at room temperature for 30 minutes and then washed.

9) The plates were then read using the Bioplex manager software, which allows for the calculation of concentrations to be done based on the generation of standard curves.

10) Concentrations for TNF-α, IL-6, ICAM-1 and VCAM-1 were expressed as ng/mL and MCP-1 as ng/L.

CHAPTER THREE: SPECTRUM OF HEART DISEASE AND RISK FACTORS IN A BLACK URBAN POPULATION IN SOUTH AFRICA: THE "HEART OF SOWETO" STUDY

Lancet. **2008; 371:915-22.**

Summary: This publication investigates the clinical range of disorders related to cardiovascular disease in patients from Soweto, presenting for the first time to a tertiary health care facility, in an attempt to improve our understanding of the characteristics and burden imposed by heart disease in an urban African community in probable epidemiologic transition.

Statement of originality document: Please see Appendix.

Spectrum of heart disease and risk factors in a black urban population in South Africa (the Heart of Soweto Study): a cohort study

Karen Sliwa, David Wilkinson, Craig Hansen, Lucas Ntyintyane, Kemi Tibazarwa, Anthony Becker, Simon Stewart

Summary

Background **The Heart of Soweto Study aims to increase our understanding of the characteristics and burden imposed by heart disease in an urban African community in probable epidemiological transition. We aimed to investigate the clinical range of disorders related to cardiovascular disease in patients presenting for the first time to a tertiary-care centre.**

Methods **From Jan 1 to Dec 31, 2006, we recorded data for 4162 patients with confirmed cases of cardiovascular disease (1593 newly diagnosed and 2569 previously diagnosed and under treatment) who attended the cardiology unit at the Chris Hani Baragwanath Hospital in Soweto, South Africa. We developed a prospectively designed registry and gathered detailed clinical data relating to the presentation, investigations, and treatment of all 1593 patients with newly diagnosed cardiovascular disease.**

Findings **Most patients were black Africans (n=1359 [85%]), and the study population contained more women (n=939 [59%]) than men. Women were slightly younger than were men (mean 53 [SD 16] years *vs* 55 [15] years; p=0·031), with 399 (25%) patients younger than 40 years. Heart failure was the most common primary diagnosis (704 cases, 44% of total). Moderate to severe systolic dysfunction was evident in 415 (53%) of 844 identified cases of heart failure, 577 (68%) of which were attributable to dilated cardiomyopathy or hypertensive heart disease, or both. Black Africans were more likely to be diagnosed with heart failure than were the rest of the cohort (739 [54%] *vs* 105 [45%]; odds ratio [OR] 1·46, 95% CI 1·11–1·94; p=0·009) but were less likely to be diagnosed with coronary artery disease (77 [6%] *vs* 88 [38%]; OR 0·10, 0·07–0·14; p<0·0001). Prevalence of cardiovascular risk factors was very high, with 897 (56%) patients diagnosed with hypertension (190 [44%] of whom were also obese). Only 209 (13%) patients had no identifiable risk factors, whereas 933 (59%) had several risk factors.**

Interpretation **We noted many threats to the present and future cardiac health of Soweto, including a high prevalence of modifiable risk factors for atherosclerotic disease and a combination of infectious and non-communicable forms of heart disease, with late clinical presentations. Overall, our findings provide strong evidence that epidemiological transition in Soweto, South Africa has broadened the complexity and spectrum of heart disease in this community. This registry will enable continued monitoring of the range of heart disease.**

Lancet 2008; 371: 915–22

See **Comment** page 876

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Introduction

The causes and consequences of an epidemic of cardiovascular disease and its major component, heart disease, in developed countries have been well documented. Conversely, few data exist in low-income and middle-income countries to describe the effect of cardiovascular disease emerging as a threat in addition to malnourishment and infectious disease,[1–3] especially in vulnerable populations for whom modifiable risk factors have previously been rare[4] and health-care resources already over burdened. The potential effect of different stages of epidemiological transition is especially evident in South Africa; this country of great diversity extends from highly industrialised cities with an urban advanced-economy lifestyle to remote rural regions with more traditional lifestyles. Although the sustained epidemic of HIV/AIDS causes 41% and 64% of deaths in men and women aged 15–44 years, respectively, coronary artery disease, hypertensive heart disease, and stroke already account for more than a third of deaths in people older than 65 years.[5,6]

We therefore established the Heart of Soweto Study[7] to monitor, describe, and respond to the evolving burden of heart disease within the largest urban concentration of black Africans in South Africa. We investigated the clinical spectrum of disorders related to cardiovascular disease (with a particular focus on heart disease) in people presenting for the first time to a tertiary-care centre.

Methods

Study setting and design

The Heart of Soweto Study is a large-scale study of emergent heart disease and its antecedents in the geographically compact townships that comprise Soweto (estimated population of 1·1 million).[7] This internationally renowned community has one of the largest urban populations of black Africans on the African continent.[7] We investigated people presenting between Jan 1, 2006, and Dec 31, 2006, for the first time to a tertiary-care centre (the Chris Hani Baragwanath

***Panel:* Sociodemographic and clinical data obtained for every new case of cardiovascular disease**

- Self-reported cultural, sociodemographic, and risk-factor profile, which included ethnic origin, length of residence in Soweto, education status, and previous clinical history
- Averaged seated systolic and diastolic blood pressure (mm Hg) and heart rate (beats per min), with measurements via a calibrated Dynamap (Critikon [GE Medical Systems Information Technologies]; Johannesburg, South Africa) monitor
- Height and weight with calculation of body-mass index (kg/m^2); data available for ambulatory patients only
- Results of an advanced cardiological assessment including heart and lung sounds and documentation of any other clinically relevant signs and symptoms on dedicated study forms
- Functional status according to the New York Heart Association (NYHA) classification of dyspnoea
- 12-lead electrocardiogram (ECG) subject to blinded coding according to published Minnesota criteria[8] to document any clinical abnormalities relating to wave-form abnormalities (eg, changes indicative of left ventricular hypertrophy), conduction (eg, left bundle-branch block), or cardiac rhythm (eg, atrial fibrillation). Data were available in 1431 (90%) patients and final determination was made by SS
- Two-dimensional targeted M-mode echocardiography with doppler colour flow mapping with a Hewlett Packard (Johannesburg, South Africa) Sonos 5500 echocardiograph attached to a 2·5 or 3·5 MHz transducer. Left ventricular dimensions and parameters (average of more than three beats) were measured according to the American Society of Echocardiography guidelines.[9] Diastolic mitral flow was assessed by pulsed-wave doppler echocardiography from the apical four-chamber view. If no abnormalities were detected during the initial echocardiographic assessment, no further specific measurements were recorded. In all other cases, echocardiographic assessment consisting of a detailed assessment of ventricular function, valvular integrity, and function and regional wall abnormalities was undertaken. For patients in atrial fibrillation, echocardiographic measures were taken at least three times and the average documented

Hospital), which provides specialist cardiac care to patients in Soweto and surrounding communities. Importantly, this 3500-bed hospital also provides referral services to individuals from a broad range of ethnic backgrounds including Europeans, Asians, and those of mixed ancestry (Khoi San European-African-Malay South Africans).

We developed a prospectively designed registry via dedicated facilities and staff (with a strong focus on local capacity building) that included all individuals presenting with confirmed or suspected cardiovascular disease to the cardiology unit. We gathered detailed clinical data relating to the presentation, investigations, and treatment of all patients with newly diagnosed cardiovascular disease. Patients with previously established cardiovascular disease were entered into a simpler version of the study's clinical registry. Data were collected for age, sex, ethnic group, and primary diagnosis for previously established cases.

The study was approved by the relevant local ethics committee and administrative bodies. The study conformed to the principles outlined in the Declaration of Helsinki. Every patient in the registry was assigned a unique identifying code (nine digits), and all documents were labelled accordingly to maintain anonymity. All participating patients provided oral consent to become part of the clinical registry.

Participants

Since the population of Soweto and surrounding communities rely on the Chris Hani Baragwanath Hospital to provide all cardiac services and treatment via the cardiology unit, clinical data from the unit's activities are highly representative of the underlying spectrum of cardiovascular disease (mild to severe). In 2006, the hospital managed 129 633 inpatients (45 400 [35%] of whom were managed by the Department of Medicine). In 2006, the case load for the cardiology unit included an estimated 5000 patients with a total of 21 000 patient contacts.

The cardiology unit is staffed by internal medicine specialists (with a minimum of 4 years' specialist training) undergoing specialist cardiology training and supported by experienced cardiologists. With gold-standard cardiological expertise and advanced diagnostic technical capacity (eg, coronary angiography and nuclear imaging), the cardiology unit provides definitive diagnostic and treatment services to the region. These services include all patients being seen at the dedicated cardiology outpatient department in addition to the hospital's coronary-care unit. The cardiology outpatient department population includes all patients seen at 12 local Soweto primary-care clinics who were referred directly for a suspected cardiac disorder; all patients requiring more definitive investigation or treatment of suspected or confirmed cardiovascular disease, who were seen initially at the general medical outpatient facilities, the specialist medical registrar clinic, and the diabetic clinic; and patients being initially admitted to the general medical or any other ward at Chris Hani Baragwanath Hospital who need a cardiological consultation.

Study data

Data were collected on a consecutive patient basis that was designed to keep selection bias to a minimum, and collection was limited only by the volume of cases at peak clinical activity. Overall, less than 10% of the administrative listing of patient case load by the cardiac-clinic clerk could not be accounted for (mainly because patients left the clinic before assessment during busy clinic days). The panel shows the sociodemographic and clinical data that the registry obtained for every new patient with cardiovascular disease.

Dependent on the clinical diagnosis and subsequent routine management of the patient, additional clinical variables that were entered into the clinical registry included: serum glucose concentration and haemoglobin A_{1c} ratio, lipid profile, full blood count (eg, platelet and white cell counts and haemoglobin), renal function (creatinine and urea concentrations), cardiac enzymes (eg, cardiac troponin concentrations), and results of HIV

testing if consent was given. In addition to the prescribed treatments, the registry also captured all advanced clinical investigative procedures (eg, coronary angiography, which was undertaken in all people diagnosed with coronary artery disease) and therapeutic procedures (eg, valve repair surgery).

All clinical diagnoses were independently reviewed by KS and SS following contemporary guidelines published online by the European Society of Cardiology[10] to finalise each individual's list of diagnoses within the clinical registry by consensus.

Statistical analysis

All study data were documented on specific study forms and then entered into a dedicated database (Microsoft Access) by the same designated data coordinator, who was a cardiac nurse with extensive clinical experience. Data were then verified (with resolution of data queries) and transferred to SAS version 9.1 for all analyses. Normally distributed continuous data are presented as the mean (SD), and variables with non-Gaussian distribution as the median (IQR). Categorical data are presented as percentages with 95% CIs when appropriate. To compare groups of patients according to demographic and clinical profile, we used χ^2 analysis with calculation of odds ratios (OR) and 95% CI for discrete variables, Student's *t* test, and analysis of variance for normally distributed continuous variables. p values were two sided.

Role of the funding source

The sponsors of the study had no role in the design of the study, data collection, data analysis, data interpretation, or the writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit the report for publication.

Results

Figure 1 shows the clinical profile of the study population. Of the 4506 patients who were assessed and entered into the registry, 344 (8%) did not have underlying cardiovascular disease: these patients had a similar sex and racial profile to those with cardiac disease, but were, on average, a decade younger (data not shown). Thus the study population consisted of 4162 confirmed patients with cardiovascular disease, 1593 (38%) of whom were newly diagnosed and 2569 (62%) were previously diagnosed and under treatment (figure 1). On average, patients with established disease were 1·7 (95% CI 0·7–2·7) years older than were those with newly diagnosed disease (p=0·0012).

The table shows the demographic and clinical profile of the 1593 patients with newly diagnosed cardiovascular disease according to their primary diagnosis. Most patients were black Africans and the study population included more women than men (table). The age profile of women was slightly younger than that for men (mean

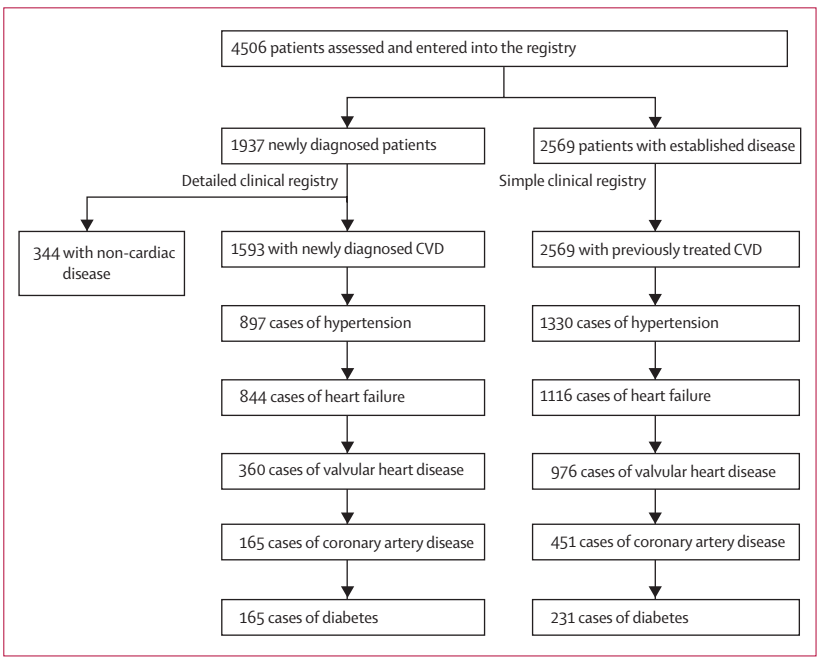


Figure 1: **Clinical profile of the study cohort**
CVD=cardiovascular disease.

age 53 [SD 16] years *vs* 55 [15] years; p=0·031), with 862 (54%) patients younger than 55 years and 399 (25%) younger than 40 years. Just over half of patients reported living in Soweto itself (table), with only 42 (5%) indicating they had lived there for less than 5 years. Black Africans were significantly more likely to report low education levels than were other races combined (381 [28%] *vs* 35 [15%]; p<0·0001).

Figure 2 shows the broad spectrum of cardiovascular disease and risk factors identified within the study cohort. Apart from patients with a primary diagnosis of hypertension, most cases represented late clinical presentations with established heart disease of more than one cause. Overall, the four most common diagnoses were hypertension, heart failure, valvular heart disease/dysfunction, and coronary artery disease (figure 2). Patients diagnosed with valvular heart disease were on average more than a decade younger than were those with heart failure or hypertension (table). Concurrent diabetes (predominantly type 2), renal disease, and anaemia were also diagnosed in some patients (table), and 74 (5%) were confirmed as HIV positive (previous diagnosis or specifically tested for the virus).

In the 146 miscellaneous cases (9% of all cases), the most common diagnosis was pericardial effusion (figure 2) as a complication due to tuberculosis, HIV/AIDS, or a combination of both diseases (n=80 [5% of all cases]). Overall, rheumatic valvular heart disease, the cardiomyopathies, and tuberculous pericardial effusion combined, accounted for 639 (40%) of these newly diagnosed cases. A clinically important but

	All (n=1593)	Hypertension (n=310)	Heart failure (n=704)	Valve disease (n=268)	CAD (n=165)	Other (n=146)
Sociodemographic profile						
Age (years)	52·8 (17·1)	58·3 (15·3)	55·1 (16·2)	45·7 (18·2)	56·7 (12·4)	38·8 (16·6)
Black African	1359 (85%)	265 (86%)	640 (91%)	243 (91%)	77 (47%)	134 (92%)
Women	939 (59%)	199 (64%)	409 (58%)	179 (67%)	68 (41%)	84 (58%)
No or standard education	416 (26%)	76 (26%)	204 (31%)	69 (27%)	29 (21%)	38 (27%)
Live in Soweto	842 (53%)	169 (55%)	418 (59%)	126 (47%)	42 (25%)	87 (60%)
Years living in Soweto	41 (27–50)	46 (35–54)	43 (30–52)	36 (20–50)	44 (40–55)	28 (17–39)
Risk factor profile						
Positive family history	405 (25%)	67 (22%)	168 (24%)	75 (28%)	60 (36%)	35 (24%)
Hypercholesterolaemia*	159 (22%)	54 (38%)	45 (17%)	16 (21%)	37 (35%)	7 (20%)
History of smoking	661 (41%)	112 (36%)	327 (46%)	84 (31%)	84 (51%)	54 (37%)
Risk factors						
None	209 (13%)	0	71 (10%)	78 (29%)	8 (5%)	52 (36%)
One	451 (28%)	72 (23%)	203 (29%)	77 (29%)	40 (24%)	59 (40%)
More than one	933 (59%)	238 (77%)	430 (61%)	113 (42%)	117 (71%)	35 (24%)
Clinical presentation						
NYHA class III or IV	486 (31%)	84 (27%)	255 (36%)	63 (24%)	32 (19%)	52 (36%)
Heart rate (min)	86 (21·5)	82 (19·2)	90 (20·3)	83 (18·3)	77 (19·2)	97 (37·6)
Systolic BP (mm Hg)	130 (27·1)	144 (29·0)	129 (26·1)	123 (24·4)	124 (27·1)	120 (23·4)
Diastolic BP (mm Hg)	73 (16·6)	78 (16·8)	75 (16·7)	68 (15·0)	70 (13·5)	71 (17·3)
Angina pectoris/chest pain	451 (28%)	89 (29%)	182 (26%)	70 (26%)	82 (50%)	28 (19%)
Oedema (pulmonary/peripheral)	494 (31%)	78 (25%)	275 (39%)	75 (28%)	35 (21%)	31 (21%)
Co-morbidity						
Renal dysfunction†	115 (10%)	23 (10%)	51 (10%)	20 (8%)	16 (11%)	5 (5%)
Anaemia‡	156 (13%)	30 (12%)	64 (11%)	22 (12%)	7 (6%)	33 (28%)
Diabetes	165 (10%)	41 (13%)	66 (9%)	13 (5%)	35 (21%)	10 (7%)
HIV positive	74 (5%)	4 (1%)	35 (5%)	10 (4%)	2 (1%)	23 (16%)
ECG profile§						
Sinus rhythm	1321 (92%)	267 (98%)	574 (90%)	222 (90%)	140 (98%)	118 (93%)
Atrial fibrillation	102 (7%)	12 (4%)	48 (8%)	32 (13%)	4 (3%)	6 (5%)
Arrhythmia	487 (34%)	79 (29%)	252 (40%)	74 (30%)	31 (22%)	51 (40%)
Bundle block	127 (9%)	11 (4%)	76 (12%)	23 (9%)	11 (8%)	6 (4%)
Echocardiographic profile§						
Mean LVEF	53·0 (17·4)	65·0 (10·8)	45·7 (18·3)	57·1 (13·4)	51·6 (15·9)	61·8 (8·8)
Systolic heart failure¶	415 (29%)	0	341 (52%)	37 (14%)	37 (31%)	0
Preserved heart failure¶	373 (26%)	0	316 (48%)	35 (13%)	22 (18%)	0

Data are number (%), mean (SD), or median (IQR). CAD=coronary artery disease. NYHA=New York Heart Association. BP=blood pressure. LVEF=left ventricular ejection fraction. ECG=electrocardiogram. *Hypercholesterolaemia defined as fasting total cholesterol serum concentration greater than 5·5 mmol/L (clinical data collected in 728 cases). †Renal dysfunction defined as a serum creatinine concentration greater than 160 µmol/L (clinical data in 1182 cases). ‡Anaemia defined as haemoglobin concentration less than 110 g/L in men and less than 100 g/L in women (clinical data in 1185 cases). §Echocardiographic data were available in 1431 and 1433 cases, respectively. ¶Systolic or preserved (diastolic) heart failure defined by the presence or absence of a left ventricular ejection fraction of 45% or less (measured on presentation to the cardiology unit) when clinical symptoms or signs of heart failure were present.

Table: **Sociodemographic and clinical profile according to primary diagnosis**

rare diagnosis was stroke. Overall, 145 (9%) patients reported a family history of stroke but only 64 (4%) were diagnosed with an acute stroke (16 cases) or as a secondary contributor to their presentation.

Black Africans accounted for most cases in all major diagnostic groups—eg, 739 of 844 (88%) cases of heart failure were in black African patients. Black Africans were significantly more likely to be diagnosed with heart failure than were the rest of the cohort (739 [54%] *vs* 105 [45%]; OR 1·46, 95% CI 1·11–1·94; p=0·009). However, they were far less likely to be diagnosed with coronary artery disease than were all other racial groups (77 [6%] *vs* 88 [38%]; OR 0·10, 0·07–0·14; p<0·0001). Although the overall proportions of men and women diagnosed with hypertension and diabetes were much the same, proportionately more women were diagnosed with valvular heart disease than were men (240 [26%] *vs* 120 [18%]; OR 1·30, 1·11–1·52; p=0·001). By contrast,

proportionately fewer women were diagnosed with heart failure (478 [51%] *vs* 366 [56%]; OR 0·89, 0·79–0·99; p=0·047) or coronary artery disease (68 [7%] *vs* 97 [15%]; OR 0·66, 0·58–0·77; p<0·0001); in absolute terms, however, 112 more women than men were diagnosed with heart failure.

On presentation, many patients had evidence of advanced disease with significant dyspnoea (New York Heart Association class III or IV), chest pain or angina pectoris, and peripheral oedema as common presenting symptoms across all diagnostic groups (table). Electrocardiographic (ECG) and echocardiographic findings were indicative of a cohort with predominantly advanced and complex disease, with 487 (34%) patients having tachycardia, or underlying cardiac dysfunction or structural disease. Of the 844 cases of heart failure, 203 (24%) had a mixed underlying aetiology, 332 (39%) had developed valvular dysfunction (eg, secondary to mitral regurgitation), and 67 (8%) had a primary diagnosis of valve disease (eg, predominantly rheumatic heart disease or degenerative valve disease) (figure 2). The three most common forms of heart failure were the dilated cardiomyopathies (35% [95% CI 32–38]), which included peri-partum cardiomyopathy, heart failure second to hypertensive heart disease (33% [30–36]), and right heart failure (27% [24–30]), which was commonly associated with underlying cor pulmonale (99 [44%] patients) (figure 2). Overall, 415 (53%) of patients with heart failure had moderate to severe systolic dysfunction and 225 (27%) had impaired diastolic function; mean left ventricular ejection fraction being 34% (SD 13) in those with dilated cardiomyopathy and 39% (SD 15) in those with ischaemic cardiomyopathy. Patients with heart failure related to underlying hypertensive heart disease (157 [56%] of 281 cases) and valvular disease (24 [36%] of 67 cases) were most likely to have impaired diastolic function. Similarly, 733 (97%) of 756 12-lead ECGs in patients diagnosed with heart failure had some form of abnormality, with 115 (16%) patients having ECG evidence of left ventricular hypertrophy.

In all diagnostic groups, the rate of common risk factors for cardiovascular disease was very high; overall, only 209 (13%) had no risk factors, whereas 933 (59%) had several risk factors. For example, 70 (47%) patients with a primary diagnosis of hypertension were also obese and 84 (51%) with coronary artery disease had a history of smoking. In those for whom we recorded body-mass index (BMI) data (ambulatory cases), women were significantly heavier than were men (mean BMI 29·6 [SD 7·7] kg/m² *vs* 25·2 [5·9] kg/m²; p<0·0001).

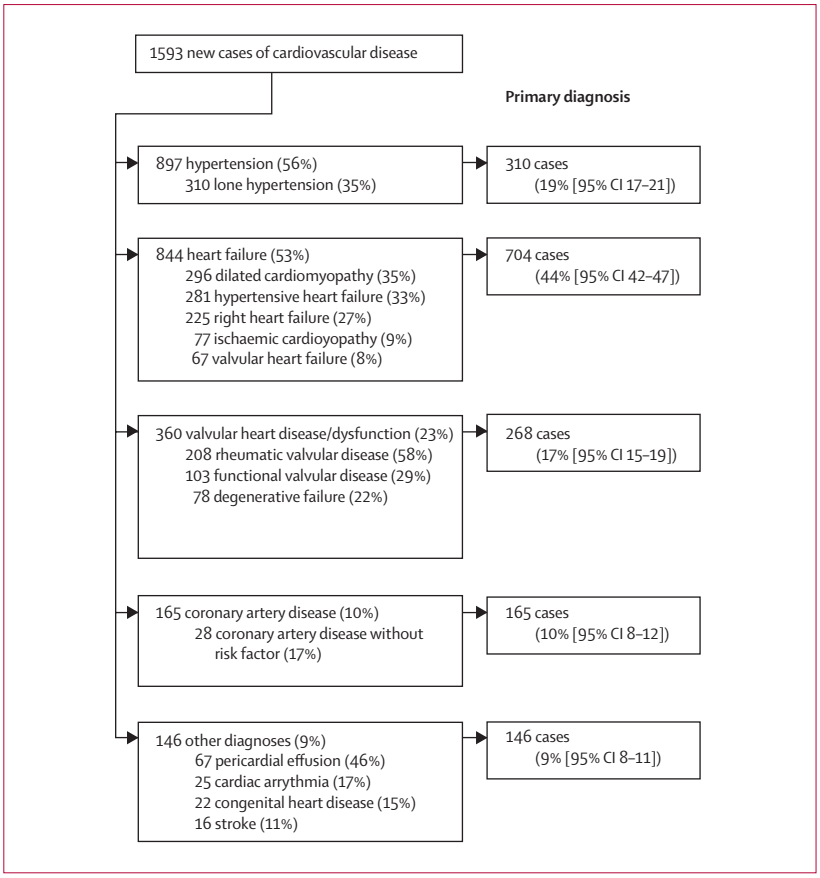


Figure 2: **Clinical spectrum of disease**

Discussion

Consistent with a call for the development of high quality, contemporaneous data to combat the global effect of cardiovascular disease,[11] this first report from the Heart of Soweto Study provides a detailed perspective on the spectrum of heart disease arising from a large urban African community. We noted that heart failure was the most common primary diagnosis in this population, with moderate to severe systolic dysfunction evident in around half the cases. Black Africans were more likely to be diagnosed with heart failure than were others, but far less likely to be diagnosed with coronary artery disease. Almost two-thirds of patients had multiple risk factors for cardiovascular disease.

Our data have important public-health and clinical implications for the prevention and treatment of heart disease both within this internationally renowned community and possibly for other urban communities on the African continent that are undergoing epidemiological transition. Specifically, we note that the present spectrum of heart disease in Soweto ranges from the so-called traditional forms of infectious diseases that are usually expected in African populations[12–14] to newer non-communicable diseases (predominantly associated with advanced clinical presentations) that are often reported in high-income countries.[15,16]

These baseline data now provide us with the opportunity to establish whether epidemiological transition in Soweto has broadened the spectrum of clinical cardiovascular disease beyond the traditional threats of rheumatic valvular heart disease, the cardiomyopathies, and

tuberculous pericardial effusion (affecting 40% of patients in 2006).[12] The presence of a large component of non-communicable cardiovascular disease (eg, hypertensive heart disease and coronary artery disease) and its common antecedent, type II diabetes, is consistent with a broadening spectrum of cardiovascular disease in this urban population.[17] In view of its near historical absence in black Africans,[7,18] the number of documented cases of atherosclerotic disease (14% overall including stroke cases) is consistent with our recent community-based survey that showed a high prevalence of modifiable risk factors in black African adults living in Soweto.[19] The clinical spectrum of heart disease within this population is further broadened by cardiac complications relating to tuberculosis and HIV/AIDS (eg, tuberculous pericardial effusion, HIV-cardiomyopathy, and diseases related to highly active antiretroviral therapy). The burden of patients presenting for the first time with symptoms of chronic rheumatic heart disease and idiopathic cardiomyopathy still exceeds the number of patients presenting with coronary artery disease. Importantly, these data also show that many patients had developed significant clinical disease before their first presentation (suggesting little awareness of heart disease and difficulty in accessing appropriate health care). Almost half of people being treated for hypertension in the absence of clinical heart disease were obese. That black African women were most likely to be obese both in this hospital cohort and the general community,[19] and that they are also the predominant sex within this ethnic cohort, is especially noteworthy in view of the typical male dominance seen in cohorts from developed countires.[15,16] Similarly, the fairly young age of the cohort also contrasts substantially with reports from high-income countries.

For decision-making and planning processes in health care, a consistent and comparative description of the spectrum and burden of diseases and their associated risk factors is essential. All broad indications, whether specific to the region[6,20] or derived from a range of developing countries,[21] indicate an increasing burden imposed by cardiovascular disease. For example, a South African survey of 10 000 households has identified a worsening risk profile for cardiovascular disease in the country.[6] Similarly, a report from Columbia University's Earth Institute showed that cardiovascular mortality rates in working-age people in South Africa were 1·5 times higher than were those of working-age people in the USA, and 41% of cases occurred in those aged 35–64 years.[20] However, probable error rates relating to estimates of all-cause mortality (range 15–20%) and prevalence of ischaemic heart disease (25–35%) for sub-Saharan Africa are substantial.[22] Few data for the spectrum and characteristics of heart disease and other major forms of cardiovascular disease in Africa exist.[11–13,23] Many studies were undertaken before echocardiography was used as standard, or many cases did not use this technique. By contrast with our report, studies usually focus on a particular disease and only seldom investigate the entire spectrum of cardiovascular disease.[13] Overall, comprehensive clinical data supported by results of appropriate investigations (ECG, echocardiography, and biochemical studies) are not available from sub-Saharan African, and our study addresses this important gap.

We noted that most patients presented with advanced disease and the most frequent primary diagnosis was heart failure. The likely reasons for these findings are complex. An absence of screening programmes for rheumatic or congenital heart disease in schoolchildren means that many individuals will present late with symptoms of heart failure in adulthood. This problem could be addressed with the emergence of portable technologies that provide cost-effective and pragmatic options for screening when resources are scarce. Similarly, underlying hypertension and diabetes are often identified only when significant end-organ damage has occurred. Little community awareness of the signs and symptoms of advanced heart disease undoubtedly contributes to a systematic delay in health seeking behaviours and patterns of referral. The disease profile is clearly different to the so-called traditional spectrum of disease; our data identified a large burden of newly diagnosed patients with rheumatic heart disease but not one case of acute rheumatic fever, which was previously a common disease.[24,25] Although this finding potentially shows improved living circumstances and access to antibiotic therapy for throat infections in children living in Soweto, it could also indicate an underestimation of acute rheumatic fever by parents or first-line health-care workers. Moreover, our data might not be representative for rural regions or other sub-Saharan countries (eg, the prevalence of rheumatic heart disease in Mozambique was reported to be 2·3 cases per 1000 on the basis of clinical screening and 30·4 cases per 1000 on the basis of echocardiographic screening).[26]

Consistent with data from other parts of Africa suggesting a broadening pattern of cardiovascular disease involving a component of greater burden imposed by atherosclerotic disease,[27,28] we recorded a small but potentially significant number of cases of coronary artery disease. Our data need careful examination in view of a cost-effectiveness analysis on the application of multidrug regimens for the primary and secondary prevention of cardiovascular disease from the perspective of six developing World Bank geographical regions, which favoured populations with high absolute risk for future cardiovascular events.[29] Our data would lend support to the application of this form of strategy on a primary prevention basis in sub-Saharan Africa, but not (as of yet) on a secondary prevention basis. More applicable, perhaps, would be culturally adapted programmes for the management of chronic diseases, particularly in

relation to the management of heart failure.[30] Clearly, these issues need to be addressed via appropriately designed and powered clinical trials in addition to sustainable community programmes for screening and awareness.

Notwithstanding a range of issues (including few health-care resources, the complexity of disease states, and need to build local research capacity), we support a call for an appropriate research agenda to better understand and respond to the evolving burden of cardiovascular disease in Africa on the basis of our findings.[13] Further to this study and other substantive attempts to investigate changes in the lifestyles and cardiovascular risk in African children,[31] similar reports from other parts of Africa are needed. We plan to continue this registry to monitor potential changes in the spectrum of cardiovascular disease presentations over the next decade.

Our study has several limitations. First, since not all patients being managed by the cardiology unit were captured by our registry, selection bias might exist. Furthermore, our focus on patients being managed by a tertiary centre to describe the spectrum of disease within the region could also have been biased. We acknowledge that individuals with subclinical disease, milder forms of cardiovascular disease, or those suffering sudden fatal events (eg, fatal haemorrhagic stroke or myocardial infarction) would not be captured by our registry; the few cases of stroke could partly be explained by the fact that haemorrhagic strokes diagnosed via CT scans with an obvious non-cardiac origin are often not referred to a cardiology centre in South Africa. Alternative cardiac services for those living in Soweto and surrounding communities are few, and the use of private facilities in black South Africans is very low.[32] Similarly, since the registry was based on routine clinical practice (albeit under the direction of trained cardiologists), we did not capture identical clinical data for all patients and have relied on clinical diagnoses. However, our ability to provide comprehensive 12-lead ECG and echocardiographic data for most patients is a major strength of our study, and wherever possible, we have adhered to the recently published STROBE guidelines relating to the reporting of this type of study.[33]

Despite some important limitations, our data provide preliminary evidence to show the effect of epidemiological transition in this population who face many threats to their present and future cardiac health, including a high prevalence of modifiable risk factors for atherosclerotic disease, a combination of infectious and non-communicable forms of heart disease, and late clinical presentations. The combination of common preventable risk factors and late clinical presentations (especially heart failure) represents a particular challenge to improve primary and secondary prevention strategies to not only reduce the number of new cases of cardiovascular disease but also improve health outcomes for those with pre-established disease. Long-term surveillance systems are also needed to monitor the success or failure of such initiatives.

Contributors

KS, DW, LN, KT, AB, and SS participated in the original design of the study. KS supervised the collection of data, and LN, KT, and AB assisted in the collection of data. KS led the writing of the report, which was co-led by DW and SS, and assisted by all other authors. CH coordinated data collection and data reports. All authors assisted in the interpretation of the study data and have seen and approved the final version of the report.

Conflict of interest statement

We declare that we have no conflict of interest.

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CHAPTER FOUR: EMERGING EPIDEMIC OF CARDIOVASCULAR DISEASE AMONG URBAN AFRICANS: ACUTE CORONARY SYNDROME AT BARAGWANATH HOSPITAL, SOWETO

***SA Heart* Spring 2006**

Summary: This publication, the second of the epidemiological studies, presents data on the presentation of acute coronary syndromes in modern Soweto compared to a study carried out in the same hospital between the years 1975 and 1980. A comparison is made in order to quantify the magnitude of change in the presentation of acute coronary syndromes in Soweto over the past 25 years.

Statement of originality document: Please see Appendix.

Emerging epidemic of cardiovascular disease among urban Africans:

Acute coronary syndrome at Baragwanath Hospital, Soweto

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ABSTRACT

Objectives:
To describe the recent increase in acute coronary syndromes among urban black South Africans in Soweto.

Design:
Cross-sectional study in 2004 and comparison with data from 1975-80.

Setting:
Chris Hani Baragwanath Hospital, Soweto.

Participants:
Patients admitted with a diagnosis of troponin positive acute coronary syndrome (ACS) in 2004, to Coronary Care Unit (CCU) and patients admitted with a diagnosis of acute myocardial infarction (AMI) in 1975-80.

Results:
We identified 154 patients with ACS in 2004. Of these 64 (42%) were black, 50 (32%) were white and 40 (26%) Asian. Between 1975 and 80, 54 patients were diagnosed with AMI (50 were black). Mean age in 2004 was 56 years and most were men (110; 71%). Risk factor prevalence was high in 2004. Black patients tended to be younger, less likely to have diabetes than Asians, and much less likely to have a family history of coronary artery disease. Black patients were more likely to have only one coronary artery affected by atherosclerosis than were white patients (48% vs 26%, p=0.07). Eight patients died in 2004 (5%) and eight in 1975-80 (15%).

Conclusion:
The annual incidence of ACS among black Africans has increased rapidly in Soweto. There seem to be significant differences in risk factor profile and extent of coronary arteries involved among the different ethnic groups studied. Continued emergence of coronary artery disease implies a significant additional burden to the health system.

KS and CA conceived of the study, supervised data collection, and drafted the paper. CA, LN and AB collected data. DW and SS contributed to data analysis and paper drafting. EL analysed data. All authors reviewed the final paper.
No funding was used to support this study.

Introduction

The burden and spectrum of non-communicable diseases differs considerably between Africa and more developed countries. Doctors working in rural African settings observe the virtual absence of coronary artery disease. The frequency of ischaemic heart disease has been documented as being very low among black South Africans[1] with an average of three patients each year admitted to Baragwanath Hospital, Soweto, South Africa with acute myocardial infarction (AMI) in the 1950s. However, it is now widely recognised that, in association with urbanisation and its associated epidemiological transition,[2,3] Africa is facing a significant burden of non-communicable diseases, many of which act as risk factors for cardiovascular disease. It has been suggested that the prevalence of coronary artery disease is increasing in sub-Saharan Africa[4,5] but there is limited data to confirm this, and what is available suggests regional variation. Furthermore, ethnicity, a construct that encompasses both cultural (e.g. language, habits, diet) and possibly genetic factors,[2,3] is a potentially important additional factor explaining such variation.

Between 1975 and 1980 at Baragwanath Hospital, serving the (then) almost exclusively black African population of Soweto only 54 cases of AMI were reported (50 black Africans; average eight each year in a community of approximately one million in size).[6] We have recently observed a significant change in the burden and spectrum of cardiovascular disease at Baragwanath Hospital, including the increased presentation of ischaemic heart disease. The population served by the hospital has however also changed to include Africans of European and Asian ancestry. This paper characterises acute coronary syndrome (ACS) at this hospital and compares the results with a study on acute myocardial infarction carried out in the same hospital between 1975 and 1980. A comparison is made in order to quantify the magnitude of change in the presentation of ACS in Soweto over the past 25 years.

Methods

Setting

Baragwanath Hospital has 3 300 beds and is the largest hospital in the southern hemisphere, with about 100 medical admissions each day. In 1975 the hospital had 2 734 beds with an average of 70 general medical admissions daily.[6] The hospital historically served the (essentially black African) population of Soweto, reportedly comprising around one million people[6] situated in the outskirts of Johannesburg. Since the re-organisation of South Africa's health services along non-racial lines in 1994, Baragwanath now serves a mixed population, drawing Africans of Asian (Indian) ancestry from Lenasia, and Africans of European ancestry from Vereeniging. The total population served is estimated at 1.5 million, of which 1 million of these are black Africans (www.statssa.gov.za). The coronary care unit at Baragwanath Hospital now routinely cares for black, Asian and white patients.

The above-mentioned population groups in the given geographical regions are also served by a network of primary care clinics and secondary care level hospitals. However, the Department of Cardiology at Baragwanath Hospital is the only source of specialist cardiology care in the public health system in Soweto. The cardiology outpatient clinic, which cares for about 100 patients each day (20-30 new patients) sees mainly non-referred patients (hence effectively providing a primary care service), but also sees patients admitted to the medical wards with a cardiac diagnosis. Furthermore the coronary care unit in the department is the only one serving this population, as is the coronary angiography service (in the public system). Patients have access to private health care if they can afford it.[7]

The study was approved by the University of the Witwatersrand Ethics Committee. The cohort of patients studied were part of a larger group that participated in the on-going acute coronary syndrome study.

Methodology

We carried out a cross-sectional study of patients admitted to the coronary care unit (CCU) in 2004, with ACS and positive troponin T test in accordance with the current European Society of Cardiology guidelines (www.escardio.org). All patients presenting with ACS are routinely admitted to the CCU for further evaluation and treatment. This syndrome comprises the following specific diagnoses:

- **ST elevation myocardial infarction:** characterised by typical symptoms of acute myocardial ischaemia in addition to a positive serum troponin T concentration and evolving ST-segment elevation of ≥ 0.1 mV in two or more contiguous ECG leads.
- **Non-ST elevation myocardial infarction:** characterised by typical symptoms and a positive troponin T concentration, but with ECG changes indicative of acute myocardial ischaemia (i.e. ST-segment depression in two or more contiguous ECG leads).
- **Unstable angina pectoris:** characterised by typical symptoms of worsening angina pectoris or occurring at rest with ECG changes indicative of myocardial ischaemia. All patients included in the study presenting with unstable angina, ST elevation or non-ST elevation infarction needed to have a positive troponin T concentration to be included. Acute coronary syndromes share the same common pathophysiologic mechanism, namely disruption of atherosclerotic plaque with different degrees of superimposed intra-coronary thrombus and distal embolisation.[8] For all patients admitted with this diagnosis in 2004, we collected data on demographic factors (age, gender, ethnicity), cardiovascular risk factors (previously diagnosed hypertension, diabetes, hypercholesterolaemia, current smoking, family history), blood pressure and electrocardiogram, standard Bruce stress test, coronary angiography, and left ventricular angiography. All diagnoses and results of investigations were verified by a qualified cardiologist and independently reviewed by KS.

Statistical analysis

Data for this study were entered into an Excel spreadsheet. Values are expressed as means ± standard deviation and percentages. Mean values were compared using ANOVA for normally distributed data. Percentages were compared using Chi-square test. A p value less than 0.05 was regarded as significant.

Results

Characteristics

In total, 154 patients with a diagnosis of ACS were admitted to the CCU of Baragwanath Hospital in 2004. The mean age was 56 years and there were more male (110; 71%) than female patients *(Table I)*. Most patients were black (64; 42%), with 50 (32%) white and 40 (26%) Asian (Indian). The gender distribution was similar for each ethnic group.

The prevalence of risk factors was high *(Table I)* and similar in each ethnic group. However, black patients were significantly younger, less likely to have diabetes or dyslipidaemia than Asian patients, and much less likely to have a family history of coronary artery disease than either Asians or whites *(Table II)*.

Coronary disease patterns, intervention and outcomes

There were significant differences in the type and extent of coronary artery disease among these patients *(Table III)*. Black patients were more likely to have single vessel disease than were white patients (48 vs 26%, p=0.07), while the distribution of vessels affected by atherosclerosis was similar among black and Asian (Indian) patients. In all, 76 patients (53%) had PTCA and 35 (24%) had coronary artery bypass graft surgery. At index admission eight patients died in 2004 (5%) and eight in 1975-80 (15%).

Temporal trends

Between 1975 and 1980 only 54 patients were admitted to the intensive care unit of Baragwanath Hospital with a diagnosis of AMI, 50 of whom were black Africans (eighth per annum)[6] and in the 1950s an average of three patients per annum with AMI were identified.[9]

Table I: Baseline characteristics (n = 154)

Characteristic	Measure
Age (years) (SD)	56.4 (11.8)
Gender (male) (%)	110 (71%)
Smoker (n = 145) (%)	83 (57%)
Hypertension (n = 143) (%)	84 (59%)
Family history (n = 149) (%)	53 (36%)
Dyslipidaemia (n = 148) (%)	61 (41%)
Diabetes (n = 149) (%)	49 (33%)
LV dysfunction, EF < 40% (%)	39 (34%)

LV: left ventricular; EF: ejection fraction

In those years the hospital served an almost exclusively black African population. We identified 64 black Africans with acute coronary syndrome in 2004 alone *(Figure 1)*. The population of Soweto has almost certainly increased since that time and the 2001 Census determined the population to be 890 000. If we assume that all black Africans identified in our study lived in Soweto then the annual incidence of hospitalised ACS is 7/100 000. The population was estimated to be 1 million in the late 1970s, providing an annual incidence of 0.5/100 000.

Table II: Frequency of coronary artery risk factors in patients from different ethnic groups (p-value refers to the comparison between blacks vs other ethnic groups)

	Ethnicity			
Risk factor	Whites	Asian	Blacks	p-value
Number	50	40	64	
Age (years) (sd)	57.7 (11.4)	59.5 (13.1)	53.6 (10.9)	0.03
Smoker (%)	24/49(29%)	25/39 (30%)	34/57 (41%)	0.32
Hypertension (%)	33/49 (39%)	18/35 (21%)	33/59 (39%)	0.29
Diabetes (%)	15 (31%)	20 (41%)	14 (29%)	0.02
Family history (%)	26 (49%)	18 (34%)	9 (17%)	<0.0001
Dyslipidaemia (%)	22 (36%)	22 (36%)	17 (28%)	0.02

Table III: Distribution of number of coronary arteries with atherosclerotic lesions in patients from different ethnic groups

Extent of coronary artery disease	Whites	Asian	Blacks	Total
Single vessel (n,%)	12 (26%)	16 (43%)	29 (48%)	57 (40%)
Two vessel (n,%)	17 (37%)	7 (19%)	18 (30%)	42 (29%)
Triple vessel (n,%)	17 (37%)	14 (38%)	13 (22%)	44 (31%)
Total (n,%)	46	37	60	143

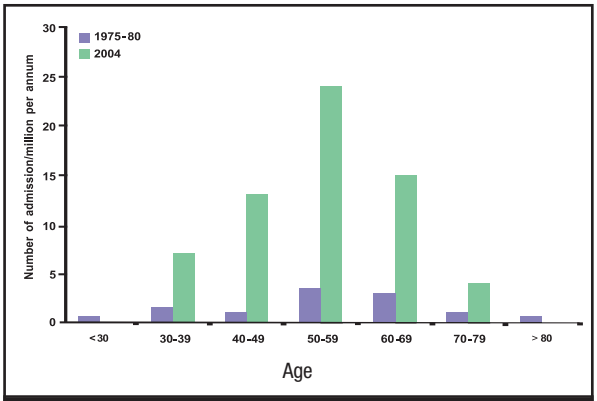


Figure 1: Comparison of the relative annual number of patients by age group presenting with ACS in 2004 and AMI during the period 1975-80, for black Africans only

However, if we assume that the population estimate in the 1970s was a significant overestimate then the annual incidence was probably higher – perhaps 1/100 000 if the population was actually 0.5 million then. Nevertheless the annual incidence seems to have increased considerably in recent years.

Discussion

The rising prevalence of cardiovascular diseases is an emergent trend in the African continent.[4,5] In this study we report on acute coronary syndromes among urban black South Africans in Soweto. Our data demonstrates a substantial increase in the number of patients diagnosed with ACS at Baragwanath Hospital, South Africa in recent years. While about 50 years ago, three such patients were identified each year,[9] and about 25 years ago, five such patients were identified each year,[6] in 2004 there were 64 black Africans with ACS. These data are important because they may herald the emergence of a significant epidemic of ischaemic artery disease in South Africa.

It is worth noting that reports from physicians working in the rural areas of South Africa have reported very low rates of ACS.[10] Anecdotal observations from one of our colleagues (DW) who had worked on stroke in the rural Agincourt area of South Africa for 12 years showed that ACS was rare as only one case was seen during the study period.[10] Such observations raise the important question of an epidemiologic transition due to urbanisation, adoption of western life style and changes from the traditional African diet to a diet high in saturated fats consistent with a population in epidemiologic transition: Is an urban epidemic of ischaemic heart disease emerging, secondary to the "westernisation" of the traditional (cardio-protective) African lifestyle in Soweto, and potentially, other urban areas?

Our data need cautious interpretation. Comparative data comes from historical publications that inevitably used different diagnostic criteria and methods which are less sensitive than current methods employed. Troponins have the greatest sensitivity and specificity in detecting myocardial infarction compared with clinical diagnosis and use of cardiac enzymes. In our study, we used very strict criteria for diagnosis of ACS in 2004 using current European Society of Cardiology guidelines and it is unlikely that our 2004 data are inflated. Although there are differences in diagnostic criteria used in our study and the two previous studies, the current data suggest an epidemiologic transition among the black population from infectious diseases to non-communicable diseases. Also, as unstable angina and AMI were so rarely diagnosed 25 and 50 years ago, and are of such clinical interest, it seems unlikely that the authors of those reports missed significant numbers of patients. However, a detailed prospective study is necessary to determine the future emergence of ACS in this population.

Overall, in 2004 we noted the typical preponderance of male cases and the high underlying prevalence of risk factors for coronary artery disease. Clearly, there were some ethnic-based differences with, for example, Asian patients demonstrating a significantly higher prevalence of diabetes. Conversely, black Africans were less likely to have a positive family history of dyslipidaemia. These observations raise two important issues. Firstly, it appears that the former likely reflects a "new" generation of coronary artery disease in the local population with the emergence of new "triggers" including the adoption of western lifestyles. Secondly, the concurrent differences in dyslipidaemia and preponderance of single vessel disease in black Africans suggest a different pathology in this group of patients. One possibility is an association with HIV infection which we are currently studying. There are reports on AMI in HIV-positive subjects[11,12] in the literature. The possible mechanisms proposed are the use of protease-inhibitor drugs which is associated with increased levels of triglycerides, total cholesterol, and low-density lipoprotein cholesterol and also its association with advanced HIV disease.[11,12] Finally we note that the mortality rate among patients with ACS at this hospital has fallen from 15% substantially to 5%, associated with the use of modern therapy including thrombolysis and PTCA.

These data are important because they may herald the emergence of a significant epidemic of ischaemic artery disease in South Africa

In conclusion, this study has highlighted a rising prevalence of coronary artery disease among South African blacks which is in keeping with the observed epidemiologic transition phase in most African countries.[4,5] Although ethnic variation in risk factors was noted, most of these factors are modifiable and efforts must be geared towards promoting a healthy life style among the general population in order to stem the impending epidemic of chronic diseases in developing countries.

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• SAHJ

Louis Vogelpoel Travelling Scholarship

Applications are invited for the first annual Louis Vogelpoel Travelling Scholarship.

An amount of up to R15 000 towards the travel and accommodation costs of a local or international congress will be offered annually by the Cape Western branch of the South African Heart Association in memory of one of South Africa's outstanding cardiologists, Dr Louis Vogelpoel.

Louis Vogelpoel was a pioneer of cardiology in South Africa who died in April 2005. He was one of the founding members of the Cardiac Clinic at Groote Schuur Hospital and University of Cape Town. He had an exceptional career over more than 5 decades as a distinguished general physician, cardiologist and horticultural scientist. His commitment to patient care, teaching and personal education is remembered by his many students, colleagues and patients. Medical students, house officers, registrars and consultants benefited from exposure to his unique blend of clinical expertise, extensive knowledge, enthusiasm and gracious style.

A gifted and enthusiastic teacher he was instrumental in the training of generations of under-graduates by regular bedside tutorials. He served as an outstanding role-model for postgraduates and many who have achieved prominence nationally and internationally acknowledged his contribution to the development of their careers.

The scholarship will be awarded for the first time in 2007 after all applications have been received and reviewed by the executive committee of the Cape Western branch of the South African Heart Association. Preference will be given to practitioners or researchers in the field of cardiovascular medicine who are members of the South African Heart Association and are resident in the Western Cape.

Applications should include:

1) a brief résumé of the work the applicant wishes to present at the congress, and
2) a brief letter of what the applicant hopes to gain by attending the relevant congress.

The applicant should submit an abstract for presentation at the relevant national or international meeting. Should such an abstract not be accepted by the relevant congress organising committee, the applicant will forfeit his or her sponsorship towards the congress. (Application can however be made well in advance of the relevant congress but will only be awarded on acceptance of the abstract). A written report on the relevant congress attended will need to be submitted by the successful applicant within six weeks of attending the congress. The congress report will be published in the South African Heart Association Newsletter.

Applications should be sent to Prof Johan Brink, President of the Cape Western branch of the South African Heart Association, Chris Barnard Division of Cardiothoracic Surgery, Cape Heart Centre, Faculty of Health Sciences, University of Cape Town, Anzio Road, Observatory, 7925 or alternatively e-mail: johan.brink@uct.ac.za.

Applications for the first round of awards close on 15 December 2006.

CHAPTER FIVE: ACUTE CORONARY SYNDROMES IN TREATMENT-NAÏVE BLACK SOUTH AFRICAN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Journal of Interventional Cardiology **2010; 23:70-77**

Summary: This publication presents data on the clinical and angiographic features of treatment-naïve HIV positive black South Africans presenting with acute coronary syndromes compared to HIV negative patients in the large urban population of Soweto, where rates of coronary artery disease have traditionally been low. This comprises the first published data on acute coronary syndromes in treatment-naïve HIV patients.

Statement of originality document: Please see Appendix.

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Acute Coronary Syndromes in Treatment-Naïve Black South Africans with Human Immunodeficiency Virus Infection

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Background: *HIV patients on protease inhibitors have greater risk of acute coronary syndromes (ACS) but little is known about treatment-naïve patients.*
Methods and Results: *Authors conducted a prospective single-center study from Soweto, South Africa, comparing the clinical and angiographic features of treatment-naïve HIV positive and negative patients with ACS. Between March 2004 and February 2008, 30 consecutive treatment-naïve HIV patients with ACS were compared to the next HIV-negative patient as a 1:1 control. HIV patients were younger (43 ± 7 vs. 54 ± 13, P = 0.004) and, besides smoking (73% vs. 33%, P = 0.002), had fewer risk factors than the control group with less hypertension (23% vs. 77%, P = 0.0001), diabetes (3% vs. 23% , P = 0.05), LDL hyperlipidemia (2.2 ± 0.9 vs. 3.0 ± 1.2, P = 0.006), and other coronary risk factors (7% vs. 53%, P = 0.0001). HDL was lower in the HIV group (0.8 ± 0.3 vs. 1.1 ± 0.4, P = 0.001). Atherosclerotic burden was lower in the HIV group with more normal infarct-related arteries (47% vs. 13%, P = 0.005) but a higher degree of large thrombus burden (43% vs. 17%, P = 0.02). Stents were used to a similar degree in HIV and control patients (30% vs. 37%, P = 0.78) with more target lesion revascularization in the HIV group (56% vs. 0%, P = 0.008).*
Conclusion: *Treatment-naïve HIV patients with ACS are younger and have fewer traditional risk factors than HIV-negative patients. HIV patients have less atherosclerotic but higher thrombotic burden which may imply a prothrombotic state in the pathogenesis of ACS in these patients.* (J Interven Cardiol 2010;23:70–77)

Background

South Africa is a country in epidemiologic transition facing an epidemic of cardiovascular disease.[1] The Heart of Soweto Study[2] provided a detailed perspective on the spectrum of heart disease arising from the large urban community of Soweto, South Africa, ranging from the so-called traditional forms of infectious diseases to newer non-communicable diseases, with a high prevalence of modifiable risk factors often reported in high income countries.[3,4] Rates of ischemic heart disease in the predominantly black population of Soweto, which have traditionally been low, are on the increase,[5] and this "double burden" of disease is particularly concerning considering the current human immune deficiency virus (HIV) pandemic with an estimated 5.5 million South Africans infected and five hundred thousand HIV-related deaths annually.[6] While cardiac complications of HIV infection are well described,[7] controversy still exists as to whether HIV seropositive subjects have a higher incidence of acute coronary syndromes (ACS). Autopsy studies in HIV-positive patients have shown unexpectedly high rates

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of atherosclerotic coronary artery disease (CAD) compared to aged-matched HIV-negative patients.[8]

The phenomenon of HIV-related premature CAD and ACS can be directly attributed to the virus- and/or treatment-related factors. Theoretically, HIV infection itself may independently predispose to premature atherosclerosis through endothelial dysfunction,[9] a heightened proinflammatory state[10,11] and dyslipidemia characterized by reductions in high density lipoprotein (HDL) cholesterol and elevations in triglycerides.[12,13] HIV patients are also prone to various abnormalities of the coagulation and fibrinolytic systems resulting in a prothrombotic state.[14–16] Alternatively, protease inhibitors (PIs) as part of highly active antiretroviral therapy (HAART) have the potential to induce an adverse metabolic phenotype including dyslipidemia and insulin resistance, endothelial dysfunction, and a prothrombotic state.[7,15,17] Following an earlier report suggesting a strong link between HAART and premature CAD,[18] the subsequent evidence has been conflicting.[7] It seems, however, that the risk of ACS is increased by prolonged exposure to PIs.[19] The historical lack of data for HAART-naïve patients presenting with concurrent HIV and CAD has, therefore, hindered attempts to understand the fundamental role of HIV status (and by its very absence HAART) in the development of premature CAD.

Study Aims

It is within this context that we have taken the unique opportunity to study a HAART-naïve population in Soweto at historically low risk for CAD to determine whether their risk factors, clinical presentation, and coronary angiographic features differ from the parallel HIV-negative population. We hypothesized that the risk profile and clinical characteristics of HIV-positive patients presenting with ACS would markedly differ from their HIV-negative counterparts, particularly in respect to the underlying morphology of coronary lesion(s) and thrombus formation.

Methods

Study Design and Patient Enrollment. We conducted a prospective single center study at Chris Hani Baragwanth Hospital, Soweto. The protocol was approved by the ethics committee of the University of the Witwatersrand and adheres to the Declaration of Helsinki. All patients gave informed consent before study entry. Between March 2004 and February 2008, all presenting patients with ACS were screened for HIV and 30 consecutive HIV-positive patients were enrolled. For each HIV patient with ACS we selected the first presenting non-HIV patient with ACS as a case-control comparator. Consistent with current guidelines,[20,21] ACS was defined as either ST elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI), or unstable angina (UA). Myocardial infarction (MI) was diagnosed in patients with compatible symptoms with elevated troponin T measurements. Patients with persistent ST elevation >0.1 mV in two contiguous leads were categorized as having STEMI and those without this criterion as having NSTEMI. Unstable angina was diagnosed in patients hospitalized with worsening angina or new-onset angina at rest without troponin T elevations. HIV was diagnosed with the standard enzyme-linked immunosorbent assay and Western blot techniques after obtaining consent and offering pretest counseling. Plasma HIV RNA level was determined by quantitative polymerase chain reaction. CD4 count was determined by flow cytometry. Conventional risk factors for arterial thrombosis were documented including a history of opportunistic infections and/or HIV-related malignancies and CDC disease stage[22] in the HIV group. The CDC HIV staging classification uses two parameters: Clinical category [A. Asymptomatic HIV infection; B. Non-AIDS defining symptomatic conditions; C. AIDS defining conditions] and CD4 count (cells/mL^3) [1. $\geq$500; 2. 200–499; 3. $<$200]. Patients were categorized as having diabetes, hypertension, or dyslipidemia when being treated chronically for these conditions or when diagnosed with the condition on admission. Patients were classified as having "other" coronary risk factors if any of the following conditions were present: (i) Family history of premature CAD (men $<$55 years, women $<$65 years), (ii) chronic kidney disease (CKD), (iii) postmenopausal state, and (iv) abdominal obesity (abdominal circumference $>$ 102 cm in men and 88 cm in women). "Multiple" risk factors was defined as $\geq$2 of: hypertension, diabetes mellitus, hyperlipidemia, smoking, or family history. The TIMI (thrombolysis in myocardial infarction) risk score for STEMI[23] and NSTEMI / UA[24] were calculated in all patients. Anthropometric measurements including body mass index (BMI), waist-to-hip ratio, and abdominal circumference were measured according to guidelines from

the InterHeart study.[25] Patients were managed according to standard ACS guidelines.[20,21] The results of all diagnostic angiograms and percutaneous coronary interventions (PCI) were assessed by two independent interventional cardiologists (AE and CZ) blinded to the patients' HIV status. Angiographic follow-up was planned for all patients receiving a coronary stent at a minimum of 6 months postprocedure. Single vessel disease was defined as a single major epicardial coronary artery with a stenosis $\geq$50% and multivessel disease $\geq$2 major epicardial arteries with stenoses $\geq$50%. The infarct-related artery (IRA) was defined by reviewing each patient's angiogram, electrocardiogram, and/or echocardiogram. An artery was considered to be infarct related if an obvious thrombus or ruptured plaque was present, or if two of the aforementioned diagnostic tests implicated the same coronary territory. The IRA was defined as angiographically normal if the contour was smooth with no angiographic features of atherosclerotic disease in any of the coronary arteries. IRA thrombus burden, when present, was classified angiographically as small or large according to a previously published descriptive model.[26] Initial TIMI flow grade in the IRA was defined as previously reported.[27] Successful PCI was defined as achievement of normal (TIMI 3) coronary flow with a residual stenosis of $\leq$ 50% and no periprocedural complications. Stent thrombosis was defined according to the Academic Research Consortium criteria.[28] Binary angiographic in-stent restenosis was defined as a $>$50% diameter stenosis at follow-up. In-stent restenosis was further defined as focal or diffuse according to a previously proposed classification.[29] Target lesion revascularization (TLR) was defined as any repeat revascularization of the IRA involving the stent and/or its 5 mm proximal or distal edges. Major adverse cardiovascular events (MACE) was defined as death, non-fatal myocardial infarction, or TLR. All patients were followed up at the Chris Hani Baragwanath cardiac clinic.

Statistical Analysis. Statistical analysis was performed using SAS version 9.1 software (SAS, Cary, NC, USA). Normally distributed continuous data are presented as the mean ($\pm$ standard deviation), and variables with non-Gaussian distribution as the median (min-max range). Categorical data are presented as frequencies and percentages. Differences between the groups were assessed using a Student's t-test for continuous variables with normal distribution and in the case of non-normal distribution, a Mann-Whitney or Wilcoxon test. For categorical variables, a chi-squared test was performed and a Fisher exact test when necessary. Changes in TIMI flow rates pre- and post-PCI between HIV-positive and -negative groups were calculated using the Wilcoxon's rank sum test. Significance was assumed at a 2-tailed value of $P < 0.05$. Univariate logistic regression was performed to determine predictors of the variables: normal IRA, large thrombus burden and death and data presented as odds ratios (OR) with 95% confidence intervals (CI). Multivariate logistic regression was then performed but due to the small sample size we were unable to include all potential explanatory variables, which were significantly different between the groups (age, smoking, hypertension, total cholesterol, LDL cholesterol, HDL cholesterol, and "other risk factors") and so performed a limited analysis using age and smoking.

Results

Between March 2004 and February 2008, we studied 60 patients presenting with an ACS comprising 30 consecutive patients diagnosed as HIV-positive and 30 HIV-negative patients (1:1 control group) who represented the next clinical presentation of ACS. Overall, these 60 study patients represented 9% of the total caseload of 673 patients presenting with an ACS during this period.[2] There were no refusals to enter the study in either group.

HIV Profile. In the HIV-positive group, the median (range) CD4 count was 231 (30–1,356) cells/mm^3 with a median (range) viral load of 29,000 (25–700,000) RNA copies/mL. All of the HIV patients were HAART naïve. There were no HIV-related opportunistic infections or malignancies and the majority of patients, 18 of 30 (60%), had early disease being classified as either CDC stage A1 or A2 with one (3%) patient classified as stage B1. The criteria for AIDS[22] were met in 11 of 30 (37%) patients in the HIV group based on a CD4 count $<$200 cells/mL^3.

Clinical and Demographic Features According to HIV Status. The clinical characteristics of the two groups according to HIV status are summarized in Table 1. None of the patients had suffered a previous MI or received prior coronary revascularization. Although the gender distribution was similar with a male predominance in both groups, HIV patients were significantly younger. The odds of an HIV-positive patient having a history of cigarette smoking (OR 5.5, 95% CI 1.81–16.68, $P = 0.002$) was significantly higher

Table 1. Clinical Features of HIV Patients and Controls with ACS

	HIV+ve (n = 30)	HIV–ve (n = 30)	P Value
Demographic Profile			
Black African n (%)	30 (100%)	30 (100%)	1.0
Mean age (years)	43 ± 7	54 ± 13	0.0004
Men (%)	20 (67%)	18 (60%)	0.79
Coronary Risk Factors n (%)			
Smoking (%)	22 (73%)	10 (33%)	0.002
Diabetes mellitus (%)	1 (3%)	7 (23%)	0.05
Hypertension (%)	7 (23%)	23 (77%)	0.0001
Total cholesterol (mmol/L)	3.6 ± 1.0	4.6 ± 1.4	0.006
LDL cholesterol (mmol/L)	2.2 ± 0.9	3.0 ± 1.2	0.006
HDL cholesterol (mmol/L)	0.8 ± 0.3	1.1 ± 0.4	0.001
Triglycerides (mmol/L)	1.4 ± 0.8	1.1 ± 0.4	0.35
Multiple risk factors	8 (27)	18 (60)	0.0182
Other coronary risk factors	2 (7%)	16 (53%)	0.0001
Clinical Features			
Systolic/ diastolic blood pressure (mmHg)	132 ± 31/ 86 ± 22	145 ± 37/ 89 ± 18	0.18 / 0.32
Pulse rate (beats/min)	91 ± 22	91 ± 29	0.9
Body mass index (kg/m^2)	25 ± 5	28 ± 5	0.01
Waist to hip ratio	0.91 ± 0.06	0.95 ± 0.05	0.03
Abdominal circumference (cm)	85 ± 10	100 ± 15	0.0003

Data are presented as the mean ± standard deviation or proportions.

than control patients. Alternatively, HIV-positive patients were less likely to have a history of other traditional risk factors for ACS including hypertension (OR 0.009, 95% CI 0.03–0.31, P = 0.0001), diabetes mellitus (OR 0.11, 95% CI 0.01–0.99, P = 0.05), elevated LDL cholesterol (2.2 ± 0.9 mmol/L vs. 3.0 ± 1.2 mmol/L, P = 0.006) and other risk factors (OR 0.06, 95% CI 0.01–0.31, P = 0.0001). HDL cholesterol was significantly lower in the HIV-positive group (0.8 ± 0.3 vs. 1.1 ± 0.4, P = 0.001). Overall 8/30 (27%) and 18/30 (60%) of HIV-positive and control patients had multiple risk factors (OR 0.24, 95% CI 0.08–0.72, P = 0.018). None of the HIV patients had a history of drug abuse but one patient in the HIV-negative group admitted to using cocaine. The HIV group had lower BMIs and AC measurements. The ratio of STEMI (77% vs. 60%, P = 0.27) to NSTEMI / UA (23% vs. 37%, P = 0.40) in the HIV group vs. the control group was similar as were the TIMI risk scores (STEMI: 3.7 ± 2.3 vs. 4.1 ± 1.9, P = 0.24; NSTEMI: 2.0 ± 0.8 vs. 2.8 ± 0.8, P = 0.06). In terms of in-hospital medication, all patients received a 300-mg loading dose of aspirin followed by 150 mg daily thereafter and a weight-adjusted antithrombotic (unfractionated heparin or low molecular weight heparin). Twenty (67%) HIV patients received clopidogrel on admission (300 mg loading dose and 75 mg daily) compared to 13 (43%) controls (P = 0.10). Thrombolytic therapy was utilized as an initial strategy in 9 (30%) HIV patients compared to 5 (17%) controls. Glycoprotein 2b3a inhibitors were used during PCI in 4 (13%) HIV patients and 7 (23%) controls (P = 0.33). Beta-blockers and statins were used in 25 (83%) patients in each group with angiotensin converting enzyme—inhibitors being utilized in 19 (63%) and 22 (73%) of HIV and control patients, respectively (P = 0.36).

Initial Angiographic Features. Coronary angiography was performed in all patients (Table 2). There was a trend toward a lower number of affected coronary vessels in the HIV group (1.3 ± 0.6 vs. 1.6 ± 0.8, P = 0.08) and toward the LAD being the most commonly involved IRA (73% vs. 50%, P = 0.06). An angiographically normal IRA was significantly more common in the HIV group and associated with thrombus in 8 of 30 (27%) compared to 2 of 30 (7%) controls (P = 0.04) (Figs. 1 and 2). TIMI 0 flow in the IRA (i.e., totally occluded) at presentation was found more commonly in the HIV-negative group (60% vs. 23%: OR 0.20, 95% CI 0.07–0.62, P = 0.004). Large thrombus burden in the IRA was seen more frequently in HIV patients (43% vs. 17%, P = 0.02). In addition, 3/30 (10%) patients in the HIV group had thrombus in a coronary artery other than the IRA compared to 0/30 (0%) control patients (P = 0.24). On univariate

Table 2. Angiographic Features of HIV Patients and Controls with ACS

	HIV+ve (n = 30)	HIV−ve (n = 30)	P Value
Diagnostic angiogram n (%)	30 (100)	30 (100)	1.0
Single vessel disease	24 (80)	18 (60)	0.08
Mean no. of affected vessels	1.3 ± 0.6	1.6 ± 0.8	0.08
Angiographically normal IRA	14 (47)	4 (13)	0.005
Thrombus in multiple arteries	3 (10)	0 (0)	0.24
Large thrombus burden	13 (43)	5 (17)	0.02
Treatment n (%)			
PCI (PTCA or stent)	18 (60)	18 (60)	1.0
PTCA alone	9 (30)	7 (23)	0.54
Stent (bare metal)	9 (30)	11 (37)	0.78
Medical therapy only	12 (40)	11 (37)	0.60
CABG	0 (0)	2 (7)*	0.49

Data are presented as the mean ± standard deviation or proportions. IRA = infarct related artery; PCI = percutaneous coronary intervention; PTCA = percutaneous transluminal coronary angioplasty; CABG = coronary artery bypass surgery.
*One control patient had CABG following PCI.

logistic regression, HIV infection predicted large thrombus burden (OR 3.82, CI 1.12–12.7, P = 0.029) and a normal IRA (OR 5.7, CI 1.6–20.3, P = 0.008). On multivariate logistic regression, adjusting for age and smoking, HIV infection was predictive of large thrombus burden (OR 5.7 [95% CI 1.1–28.2], P = 0.034) but not "normal IRA" (OR 3.7 [95% CI 0.8–16.5], P = 0.09). The only predictor of a "normal IRA" was age (OR 0.9 [95% CI 0.9–1.0], P = 0.017).



Figure 1. Angiographic image of thrombus in an otherwise angiographically normal left anterior descending coronary artery in a 43-year-old HIV-positive male presenting with an ST-elevation MI.

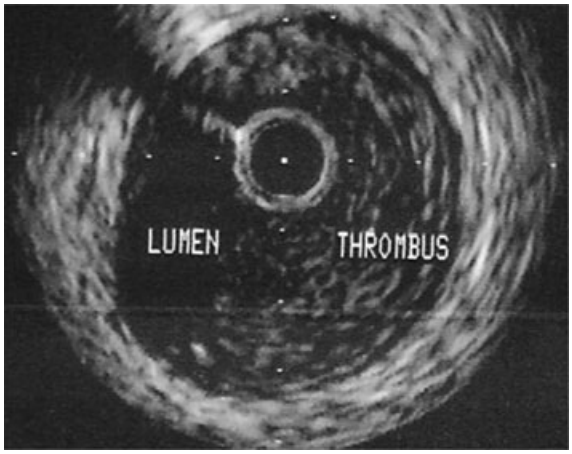


Figure 2. Intravascular ultrasound image of thrombus in an otherwise angiographically normal left anterior descending coronary artery depicted in Figure 1.

Coronary Intervention. PCI was the most frequently utilized treatment strategy in both groups with a similar usage of bare metal stents (BMS) and acute procedural success rates of 9 of 9 (100%) in the HIV group and 8 of 11 (73%) in the control group (P = 0.36). Angioplasty alone was performed in 9 of 30 (30%) HIV patients and 7 of 30 (23%) controls (P = 0.54). Pre-PCI TIMI flow scores were different between the two groups with a higher percentage of control patients having TIMI 0 flow pre-PCI (15/18 [83%] controls vs. 4/18 [22%] HIV, P = 0.0007). There was no difference in post-PCI TIMI flow scores or the change between pre- and post-PCI TIMI flow scores. PCI complications occurred in 7of 30 (23%) patients in both groups. There was one serious complication (definite sub-acute stent thrombosis) occurring in an HIV patient (1 of 9 [11%]), 4 days after receiving a BMS resulting in a non-fatal MI. Medical treatment alone was used in a similar number of HIV and control patients (12 of 30 [40%] vs. 11 of 30 [37%], P = 0.60) and 2 of 30 (7%) HIV-negative patients underwent surgical revascularization compared to none in the HIV group (P = 0.49).

Angiographic Follow-Up. Follow-up angiography was performed in 11 of 20 (55%) patients who received BMS at a mean duration of 9 months post-implantation: 5 of 9 (56%) in the HIV group compared to 6 of 11 (55%) in the control group (P = 0.38). Overall, 9 of 20 (45%) patients were unable to be studied owing to 2 deaths and 2 patients lost to follow-up in the HIV group and 3 deaths and 2 patients lost

Table 3. Long-Term Outcomes of HIV Patients and Controls with ACS

	HIV+ve (n = 30)	HIV–ve (n = 30)	P Value
Patients n (%)	30 (100)	30 (100)	1.0
Clinical Outcomes at 48 Months			
MACE	14 (47)*	5 (17)	0.026
Death	9 (30)	5 (17)	0.36
Non-fatal MI	1 (3)	0 (0)	1.0
TLR	5 (17)	0 (0)	0.05
Angiographic Follow-Up			
Patients originally receiving BMS	9 (30)	11 (37)	0.78
Patients eligible for follow-up angiography	5/9 (56)	6/11 (55)	0.38
Mean duration of follow-up angiogram (months)	7 ± 5	11 ± 3	0.14
Binary in-stent restenosis	5/5 (100)	3/6 (50)	0.18
Focal	0/5 (0)	1/3 (33)	0.75
Diffuse	5/5 (100)	2/3 (67)	
Composite TLR†	5/9 (56)	0/11 (0)	0.008

Data are presented as proportions or mean ± standard deviation.
MACE = major adverse cardiovascular events; MI = myocardial infarction; TLR = target lesion revascularization.
*One HIV patient had a non-fatal MI requiring TLR.
† TLR in patients receiving a bare metal stent.

to follow-up in the control group. Binary angiographic restenosis in patients who received a BMS with angiographic follow-up occurred in 5 of 5 (100%) HIV patients and 3 of 6 (50%) controls (P = 0.18).

In the HIV group, binary angiographic restenosis of a diffuse proliferative pattern (type 3)[29] was found in all 5 (100%) patients studied. In the control group, 2 of 6 patients (33%) had a diffuse proliferative pattern (type 4) and 1 of 6 (17%) had focal body restenosis (type 1C). Ischemia-driven TLR was performed in all 5 HIV patients with diffuse proliferative restenosis with an angioplasty in 1 of 5 (20%) patients and drug-eluting stents (DES) in 4 of 5 (80%) patients with good procedural success. No TLR was performed in the control group due to lack of objective evidence of reversible ischemia on nuclear perfusion imaging. The composite TLR rate for HIV patients and controls who received a BMS was 5 of 9 (56%) vs. 0 of 11 (0%), respectively (P = 0.008).

Clinical Outcomes. Follow-up data were available from the time of admission to last follow-up at 48 months (Table 3). MACE rates were higher in the HIV group (47% vs. 17%, P = 0.026), driven by increased rates of TLR. Nine of 30 (30%) HIV patients died compared to 5 of 30 (17%) controls (P = 0.36). Overall 3 of 9 (33%) fatal events in the HIV group occurred at the index admission (two from cardiogenic shock and one from a cardio-embolic stroke). A further 6 patients died after the index event (67% of deaths): 1 from a massive pulmonary embolus and 5 from unknown causes. In the control group, 1 of 5 fatal events (20%) occurred during the index admission due to cardiogenic shock. The remaining deaths (80%) included a fatal stroke, one recurrent MI, a suicide, and one of unknown cause.

Discussion

Taking advantage of historically low treatment rates for HIV in South Africa, we were able to document clinical presentations of ACS from the predominantly black African community of Soweto on the basis of HIV status without the potentially confounding effects of underlying HAART. As such, in one of the largest reported series of HAART-naïve HIV-positive patients presenting with ACS, we found that the majority of HIV patients did not have advanced HIV disease and were all asymptomatic prior to presentation: only 11/30 (37%) were eligible for HAART therapy according to our current guidelines making this an important group for primary prevention. Classical risk factors for ACS were more prevalent in the control group with an older population and more hypertension, diabetes, hyperlipidemia, abdominal obesity, and other risk factors. Cigarette smoking was, however, more prevalent in the HIV group and consistent with other studies.[30] HIV patients also had significantly lower HDL levels, an independent risk factor for CAD, thrombosis, and ACS.[31]

How do we interpret these results relative to previous reports? Consistent with other studies from the developed world,[30] our HIV group was younger in age and predominantly male with a high proportion of cigarette smokers and low HDL which is due, in part, to an impaired reverse cholesterol transport pathway mediated by the HIV regulatory protein nef.[32] In contrast to findings in the developed world, none of our HIV patients were intravenous drug abusers and none had opportunistic infections or HIV-related malignancies, factors known to confer a higher thrombotic risk.[16] Degree of immunosuppression did not correlate with risk of ACS. HIV patients had less atherosclerotic burden angiographically with significantly more angiographically normal IRAs probably reflecting the younger age of the group with less classical risk factors despite the potentially detrimental effects of a heightened inflammatory state. The significantly higher degree of large thrombus burden in the IRA of HIV patients suggests a prothrombotic state which is well described and probably multifactorial[14–16] in nature.

What are the potential mechanisms underlying our findings? A study by Davies and colleagues[33] regarding the causes of MI in young patients without atherosclerosis emphasized the importance of excluding the antiphospholipid syndrome as a potential cause of thrombosis in patients with angiographically normal IRAs. HIV patients are known to have a high incidence of antiphospholipid antibodies ranging from 46% to 90% in various studies[14,16,34] but the relationship to thrombotic risk is unclear and further studies are needed. PCI was the preferred method of revascularization in both groups with good procedural success rates but a high incidence of diffuse proliferative in-stent restenosis was found in HIV patients receiving BMS, in keeping with other studies,[30,35] the pathogenesis of which is thought to be due to the heightened inflammatory state at the time of PCI.[36] DES are currently the preferred treatment for in-stent restenosis requiring revascularization[37] but no data exist on their efficacy or safety in the setting of HIV infection. In this study, four HIV patients with diffuse proliferative in-stent restenosis received DES with good procedural success and no need for TLR during follow-up.

This study has a number of limitations that require comment. Although this represents a historically large cohort of HAART-naïve HIV patients presenting with ACS, the study cohort overall was small and thus underpowered to detect small differences between the groups. Although HIV status predicted a normal IRA and large thrombus burden on univariate logistic regression, the sample size was too small to allow for multivariate logistic regression using all baseline explanatory variables and thus one cannot conclude that HIV alone was responsible for these findings. Age-specific MI rates in black patients from Soweto are not known and we were thus unable to determine whether the incidence of ACS is higher in HAART-naïve HIV patients compared to age-matched HIV-negative patients. Not all patients receiving BMS underwent repeat angiography affecting the reliability of the data regarding in-stent restenosis but wherever possible we have adhered to the recently published STROBE guidelines in our reporting of study data.[38] The potential role of a hypercoagulable state including antiphospholipid antibodies in the pathogenesis of ACS in this HAART-naïve HIV group is currently being investigated.

In summary, in one of the largest studies of its type, we found that HAART-naïve, HIV-positive black South Africans presenting with ACS have different clinical and angiographic features compared to the HIV-negative population. Similar to reports on HIV patients receiving HAART,[7] our patients are younger, more commonly male with higher rates of smoking, low HDL and less atherosclerotic burden but a higher thrombotic burden suggesting a prothrombotic state which needs further investigation. The exact pathogenetic role of HIV, independent of associated risk factors, in ACS and its treatment remains unclear but may be important as a contributory factor in an already "vulnerable patient" and needs further elucidation with larger prospective studies. Importantly, we identified modifiable cardiovascular risk factors in the HIV group. Smoking may play a crucial role in the pathogenesis of ACS in these otherwise seemingly low-risk patients and remains an important target for cardiovascular risk reduction. The role of HDL in the pathogenesis and prevention of HIV-associated CAD needs to be further defined as does the role of DES in the prevention of restenosis. Cardiovascular risk assessment and appropriate primary prevention should be an important component in the management of HIV patients both pre- and post-treatment.

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CHAPTER SIX: THE THROMBOTIC PROFILE OF TREATMENT-NAÏVE BLACK SOUTH AFRICANS WITH HIV AND ACUTE CORONARY SYNDROMES

Clinical and Applied Thrombosis/Hemostasis, published **May 11 2010**

Summary: This publication attempts to answer some important questions raised in Chapter 5 regarding the pathogenesis of acute coronary syndromes in treatment-naïve black South Africans by looking, in a detailed fashion, at thrombotic parameters and their role in inducing a prothrombotic state.

Statement of originality document: Please see Appendix.

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The Thrombotic Profile of Treatment-Naive HIV-Positive Black South Africans With Acute Coronary Syndromes

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Abstract

Background: Patients with human immunodeficiency virus (HIV) infection on protease inhibitors (PIs) have a heightened risk of arterial thrombosis but little is known about treatment-naive patients. Methods/Results: Prospective study from South Africa comparing thrombotic profiles of HIV-positive and -negative patients with acute coronary syndrome (ACS). A total of 30 treatment-naive HIV-positive patients with ACS were compared to 30 HIV-negative patients with ACS. Patients with HIV were younger; and besides smoking (73% vs 33%) and low high-density lipoprotein (HDL; 0.8 $\pm$ 0.3 vs 1.1 $\pm$ 0.4), they had fewer risk factors. Thrombophilia was more common in HIV-positive patients with lower protein C (PC; 82 $\pm$ 22 vs 108 $\pm$ 20) and higher factor VIII levels (201 $\pm$ 87 vs 136 $\pm$ 45). Patients with HIV had higher frequencies of anticardiolipin (aCL; 47% vs 10%) and antiprothrombin antibodies (87% vs 21%). Conclusion: Treatment-naive HIV-positive patients with ACS are younger, with fewer traditional risk factors but a greater degree of thrombophilia compared with HIV-negative patients.

Keywords

acute coronary syndromes, blood coagulation factors, clinical thrombophilia, hypercoagulability, thrombophilia, thrombosis

Background

Hematologic abnormalities among individuals infected with the human immunodeficiency virus (HIV) are well described, and although the cytopenias are the most common, there is evidence that HIV induces a hypercoagulable state resulting in thromboembolic complications.[1] Rates of venous thromboembolic (VTE) complications are increased in HIV-positive patients receiving highly active antiretroviral therapy (HAART), with an incidence ranging from 0.26% to 7.6%.[2] Increased rates of arterial thrombosis have also been described, but the mechanism of this association remains unclear in the face of other associated cardiovascular risk factors such as smoking and dyslipidemia commonly found in HIV-positive patients.[3] The mechanisms responsible for this hypercoagulable state are thought to be multifactorial in nature (Table 1). Thrombotic complications may arise as a direct result of the virus or indirectly through associated opportunistic infections, malignancies, or the effects of HAART,[1] and the thrombotic potential seems to correlate with the degree of immunosuppression as measured by CD4 cell count as well as with the presence of opportunistic manifestations of acquired immunodeficiency syndrome (AIDS).[1] Human immunodeficiency virus causes endothelial cell and platelet activation, with elevated concentrations of cellular adhesion molecules (CAMs)[17] as well as von Willebrand factor (VWF),[8,11] all of which predict future cardiovascular events.[18,19] Abnormalities of coagulation factors include deficiencies of protein C (PC),[8] protein S (PS),[9,20] antithrombin (AT),[10] and heparin cofactor 2 (HC-II).[10] Antiphospholipid antibodies (aPL) and lupus anticoagulants (LAs) are highly prevalent in HIV-positive patients but their role in thrombosis is unclear.[14] Protease inhibitors (PIs) as part of HAART have been associated with VTE disease[15,21] and may lead to hypercholesterolemia, hypertriglyceridemia, and insulin resistance and have been associated with increased rates of coronary events.[22] Whether treatment-naive HIV-infected patients are also at higher risk of thrombosis is largely unknown. We have shown that treatment-naive

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Table 1. HIV and Risk Factors for Thrombosis[a]

Abnormality	Affected Vascular Beds		
	Venous	Venous and Arterial	Arterial
Defects in vessel wall		Chronic inflammation[4,5] Vasculitis[1]	Atherosclerosis[6] Metabolic syndrome[6] Hypertension Diabetes Dyslipidemia Smoking[7]
Defects in coagulation factors	Deficiency of PC[8] Deficiency of PS[9] Deficiency of AT[10] Deficiency of HCII[10] ↑ Microparticles[11]		
Defects in fibrinolysis		↑ PAI-1[12]	
Platelet defects		TTP[1]	↑ Platelet activation[13]
Stasis	Immobilization[1] CCF[1]		
Other	Malignancy[1] Opportunistic infection[1] Nephrotic syndrome[1] AIHA[1]	Antiphospholipid syndrome[14] Protease inhibitors[15]	

NOTES: AIHA = autoimmune hemolytic anemia; AT = antithrombin; CCF = congestive cardiac failure; HCII = heparin cofactor II; PAI-1 = plasminogen activator inhibitor-1; PC = protein C, PS = protein S; TTP = thrombotic thrombocytopenic purpura; ↑ = increase.
[a] Adapted from *N Engl J Med*. 2002;346(10):752-763.[16]

HIV-positive black South African patients presenting with acute coronary syndromes (ACSs) are younger with fewer traditional risk factors compared to HIV-negative patients and have less atherosclerotic but higher thrombotic burden on angiography, suggesting a hypercoagulable state.[23]

Study Aims

It is within this context that we report on the thrombotic profiles of this group of treatment-naive black South African patients with ACS compared to the parallel HIV-negative population. We hypothesized that the HIV-positive patients would have evidence of thrombophilia on laboratory testing.

Methods

Study Design and Patient Enrollment

We conducted a prospective single-center study in the Department of Cardiology at Chris Hani Baragwanth Hospital, Soweto, South Africa. The protocol was approved by the ethics committee of the University of the Witwatersrand and adheres to the Declaration of Helsinki. All patients gave informed consent before study entry. Between March 2004 and February 2008, 30 consecutive black HIV-positive patients presenting with ACS (ACS+:HIV+ group) were enrolled. For each HIV-positive patient with ACS, we selected the first presenting non-HIV black patient with ACS as a case–control comparator (ACS+:HIV− group). In addition, a second control group consisting of 30 asymptomatic HIV-positive patients matched for age, sex, and ethnicity (ACS−:HIV+ group) were recruited from the HIV clinic. Consistent with current guidelines,[24,25] ACS was defined as ST-elevation myocardial infarction (MI), non-ST–elevation MI, or unstable angina. Infection with HIV was diagnosed with the standard enzyme-linked immunosorbent assay (ELISA) and Western blot techniques, after obtaining consent and offering pretest counseling. In the HIV group, the plasma HIV RNA level was determined by quantitative polymerase chain reaction. CD4 count was determined by flow cytometry, and patients were staged according to the Centers for Disease Control and Prevention (CDC) staging system.[26] Patients were categorized as having diabetes, hypertension, or dyslipidemia when being treated chronically for these conditions or when diagnosed with the condition on admission. Patients were classified as having "other" coronary risk factors if any of the following conditions were present: (1) family history of premature coronary artery disease (CAD; men <55 years, women <65 years), (2) chronic kidney disease, (3) postmenopausal state, and (4) abdominal obesity (abdominal circumference >102 cm in men and 88 cm in women). Demographic data were recorded for each patient and anthropometric measurements including weight, height, body mass index (BMI), waist-to-hip ratio, and abdominal circumference (AC) were measured on admission, according to guidelines set out in the Interheart study.[27] Patients with ACS were managed according to the accepted guidelines set out by the European Heart Association[24,25] and followed up at the Chris Hani Baragwanath cardiac clinic.

Thrombophilic Screening

As part of the thrombophilic workup, all patients had aPL assays at baseline and further thrombophilic testing at 6 weeks postevent (ACS group), assessing coagulation factors, endothelial activation markers, and platelet function. The ACS−:HIV+ group had all tests performed at baseline, as this group had not suffered any thrombotic event. Blood was obtained by clean venipuncture with seated participants, and nonfasting venous blood was drawn into plastic tubes containing sodium citrate for the coagulation studies, EDTA for the full blood count and platelet analysis, and plain tubes for analysis of aPL. The samples were transported to the National Health Services Laboratory (NHLS), Johannesburg Hospital, at ambient temperature. All assays were performed according to the standard operating procedures of the NHLS laboratory. The analysis of aPL was performed at Lancet Laboratories, Johannesburg.

Laboratory Methods

Coagulation studies including international normalized ratio (INR), activated partial thromboplastin time (aPTT), PC (chromogenic assay, normal value 70-160 IU/dL), PS (functional assay, normal value 60-140 IU/dL), AT (chromogenic assay, normal value 76-125 IU/dL), fibrinogen (Clauss method, normal value 2-4 g/L),[28] factor VIII (normal value 50-150 IU/dL), and activated PC (APCR) resistance (normal value >2) were performed on an automated coagulometer (ACL; Instrument Laboratories, Milan, Italy). Lupus anticoagulants were detected using kaolin clotting time (KCT) with simplified Dilute Russell's Viper Venom Test (DRVVT) screening (LAC, Lupus anticoagulant screen) and confirmation reagent (LAC confirm; Dade Behring, Milton Keynes, United Kingdom) to confirm the presence of LAs in patients with prolonged KCT. Lupus anticoagulants were considered present if the ratio of LAC screen to LAC confirm was >1.3. Quantitative D-dimer (normal value <0.5 mg/L) and VWF antigen (VWAG, von Willebrand factor antigen, normal value 50%-150%) were measured using a turbidometric Latex immunoassay (Instrument Laboratories). von Willebrand factor activity (VWACT, von Willebrand factor activity, normal value 50%-150%) was measured by semiquantitative immunoassay (Dade Behring). Antiphospholipid antibodies including anticardiolipin (aCL), anti-β_2-glycoprotein-I (anti-β_2-GP-1), and antiprothrombin (aPT; immunoglobulin G [IgG], IgM, and IgA) were measured at baseline in all patients using commercial ELISA kits (Orgentec Diagnostika, Mainz, Germany).

Results for each of the 3 isotypes of the 3 types of aPL measured were considered positive when the optical density obtained for each patient exceeded that of the mean value plus 2 SD of the 100 sera from black South African normal healthy participants. Platelet aggregometry was performed using an APACT-2 (Automated platelet aggregation coagulation tracer-2) light transmission aggregometer (LTA; Instrument Laboratories) using arachidonic acid, adenosine diphosphate (ADP), collagen, and adrenaline as agonists according to the standard operating procedures of the NHLS. Where appropriate, platelet sensitivities were performed using decreasing concentrations of the appropriate agonist. Full aggregation responses to <0.5 mmol/L arachidonic acid, <2 μg/mL collagen, and <1 μg/mL ADP was defined as platelet hyperactivity. Aspirin resistance was defined as platelet aggregation in the presence of arachidonic acid of ≥20%.

Statistical Analysis

Statistical analysis was performed using SAS 9.1 software (SAS, Cary, North Carolina). Normally distributed continuous data are presented as the mean (±SD) and variables with non-Gaussian distribution as the median (min–max range). Categorical data are presented as frequencies and percentages. The initial analysis compared variables between the 3 groups using the 1-way analysis of variance (ANOVA) test for continuous variables with normal distribution and the Kruskal-Wallis test in case of nonnormal distribution. For categorical variables, the chi-square test was performed with a Fisher exact test when necessary. Significant differences between variables in the 3 groups were assumed at $P < .05$. Subgroup analysis with multiple pairwise comparisons was then performed applying the Bonferroni correction with a P value <.0166 considered significant.

Results

Acute Coronary Syndrome (ACS+) Group

The clinical and thrombotic profiles of the ACS+:HIV+ and ACS+:HIV− groups are listed in Tables 2 and 3, respectively.

Clinical profile. The HIV-positive patients were younger with a similar sex distribution. Besides smoking (33% vs 73%, $P = .004$), coronary risk factors were higher in the ACS+:HIV− group with more hypertension ($P = .0001$), low-density lipoprotein (LDL) hyperlipidemia ($P = .003$), diabetes mellitus ($P = .03$), and "other coronary risk factors" ($P = .0001$). The ACS+:HIV+ group had lower high-density lipoprotein (HDL) levels ($P = .0006$) and a lower mean BMI ($P = .008$).

Thrombotic profile. Thrombotic screens were performed in 21 (70%) of 30 and 24 (80%) of 30 patients in the ACS+:HIV+ and ACS+:HIV− groups, respectively. In the ACS+:HIV+ group, 9 of 30 (30%) patients were not tested owing to 6 deaths and 3 patients lost to follow-up. Of 30, 6 (20%) patients in the ACS+:HIV− group were not tested owing to 4 deaths and 2 patients lost to follow-up. Patients in the ACS+:HIV+ group had significantly lower mean PC ($P = .0003$) levels compared to the ACS+:HIV− group. Of 19, 4 (21%) patients in the ACS+:HIV+ group (2 excluded due to warfarin usage) had PC deficiency based on levels <70 IU/dL compared to 0 (0%) of 23 in the ACS+:HIV− group (1 patient excluded due to warfarin usage, $P = .06$). Factor VIII levels were also found to be significantly higher in the ACS+:HIV+ group ($P = .001$). Of 21, 14 (67%) patients in the ACS+:HIV+ group

Table 2. Acute Coronary Syndrome Group: Clinical Profile[a]

	ACS+:HIV+ (n = 30)	ACS+:HIV− (n = 30)	*P* Value
Demographic profile			
Black African, n (%)	30 (100)	30 (100)	1
Mean age (years)	43 ± 7	54 ± 13	.0002
Men (%)	20 (67)	18 (60)	NS
Coronary risk factors, n (%)			
Smoking	22 (73)	10 (33)	.004
Diabetes mellitus	1 (3)	7 (23)	NS
Hypertension	7 (23)	23 (77)	.0001
Total cholesterol (mmol/L)	3.6 ± 1.0	4.6 ± 1.4	.0031
LDL cholesterol (mmol/L)	2.2 ± 0.9	3.0 ± 1.2	.0032
HDL cholesterol (mmol/L)	0.8 ± 0.3	1.1 ± 0.4	.0006
Triglycerides (mmol/L)	1.4 ± 0.8	1.1+/-0.4	NS
BMI (kg/m^2)	25 ± 5	28 ± 5	0.008
Other coronary risk factors	2 (7)	16 (53)	0.0001

NOTES: ACS = acute coronary syndrome; BMI = body mass index; HIV = human immunodeficiency virus; NS = not significant.
[a] Data are presented as mean ± standard deviation or proportions.

Table 3. Acute Coronary Syndrome Group: Thrombotic Profile[a]

	ACS+:HIV+ Group	ACS+:HIV− Group	*P* Value
Thrombotic screen at baseline, n (%)	30 (100)	30 (100)	
Hemoglobin (g/dL)	13.6 ± 2.8	14.5 ± 1.8	NS
Platelet count ($\times 10^9$/L)	301 ± 113	302 ± 72	NS
Antiphospholipid antibody frequencies, n (%)			
Anticardiolipin (IgG)	14 (47)	3 (10)	.003
Anticardiolipin (IgM)	3 (10)	1 (3)	NS
Anticardiolipin (IgA)	0 (0)	3 (10)	NS
Anti-ß-2 glycoprotein (IgG)	11 (37)	6 (20)	NS
Anti-ß-2 glycoprotein (IgM)	3 (10)	0 (0)	NS
Anti-ß-2 glycoprotein (IgA)	7 (23)	12 (40)	NS
Antiprothrombin (IgG)	26 (87)	6 (20)	.001
Antiprothrombin (IgM)	2 (7)	0 (0)	NS
Antiprothrombin (IgA)	2 (7)	1 (3)	NS
Thrombotic screen 6 weeks post event, n (%)[b]	21 (70)	24 (80)	
Fibrinogen (g/L)	3.5 ± 1.0	3.9 ± 2.2	NS
D-Dimer quantitative (mg/L)	0.42 (0.2-2.49)	0.28 (0.2-3.08)	NS
Antithrombin (IU/dL)	101± 19	103 ± 15	NS
Protein C (IU/dL)[c]	82 ± 22	108 ± 20	.0003
Protein S (IU/dL)[c]	91± 29	107± 25	NS
APCR	2.6 ± 0.5	2.8 ± 0.5	NS
Factor 8 (IU/dL)	201 ± 87	136 ± 45	.001
VWF antigen (%)	225 ± 64	197 ± 84	NS
VWF activity (%)	108 ± 7	105 ± 12	NS
Lupus anticoagulant, n (%)	0 (0)	0 (0)	NS

NOTES: ACS = acute coronary syndrome; Ig = immunoglobulin; HDL = high-density lipoprotein; HIV = human immunodeficiency virus; LDL = low-density lipoprotein; NS = not significant; VWF = von Willebrand factor.
[a] Data are presented as mean ± standard deviation, proportions, or median (min–max range).
[b] Nine patients in ACS+:HIV+ group and 6 patients in ACS+:HIV− group were ineligible for testing at 6 weeks (see text).
[c] Patients receiving warfarin excluded from analysis (2 patients in ACS+:HIV+ and 1 patient in ACS+:HIV− group).

compared to 8 (33%) of 24 in the ACS+:HIV group had levels >150 IU/dL ($P = .07$). The ACS+:HIV+ group had higher frequencies of aCL IgG (47% vs 10%, $P = .003$) and aPT IgG (87% vs 20%, $P < .001$) compared to the HIV-negative group. There were no differences between groups with respect to the other aPL frequencies.

Platelet studies. Light transmission aggregometry was performed in 21 (70%) of 30 patients in the ACS+:HIV+ group and 24 (80%) of 30 patients in the ACS+:HIV− group. In the ACS+:HIV+ group, 16 (76%) of 21 patients were taking aspirin (150 mg/d) and 2 (10%) of 21 clopidogrel (75 mg/d). In the ACS+:HIV− group, 22 (92%) of 24 patients were taking

Table 4. HIV Group: Clinical Profile[a]

	ACS+:HIV+ (n = 30)	ACS−:HIV+ (n = 30)	*P* Value
Demographic profile			
Black African, n (%)	30 (100)	30 (100)	1
Mean age (years)	43 ± 7	41 ± 8	NS
Men (%)	20 (67)	19 (63)	NS
HIV-related factors			
CD4 (cells/mm^3)	230 (30-1356)	125 (6-1041)	.0126
Viral load (RNA, copies/mL)	29 000 (25-7 × 10^5)	54 000 (25-11 × 105)	NS
AIDS-defining criteria (CDC), n (%)	11 (37)	21 (70)	.01
Coronary risk factors, n (%)			
Smoking	22 (73)	11 (37)	.0026
Diabetes mellitus	1 (3)	0 (0)	NS
Hypertension	7 (23)	2 (7)	NS
Total cholesterol (mmol/L)	3.6 ± 1.0	3.7 ± 0.8	NS
LDL cholesterol (mmol/L)	2.2 ± 0.9	2.0 ± 0.5	NS
HDL cholesterol (mmol/L)	0.8 ± 0.3	1.0 ± 0.5	.011
Triglycerides (mmol/L)	1.4 ± 0.8	1.4+/-0.8	NS
BMI (kg/m^2)	25 ± 5	21 ± 4	.003
Other coronary risk factors	2 (7)	0 (0)	NS

NOTES: AIDS = acquired immunodeficiency syndrome; BMI = body mass index; CDC = Centers for Disease Control and Prevention; HDL = high-density lipoprotein; HIV = human immunodeficiency virus; LDL = low-density lipoprotein.
[a] Data are presented as mean ± standard deviation, proportions, or median (min–max range).

aspirin (150 mg/d) and 4 (17%) of 24 clopidogrel (75 mg/d). Patients taking aspirin or clopidogrel were analyzed separately from patients on no antiplatelet drugs. Of the patients taking aspirin, 3 (17%) of 16 patients in the ACS+:HIV+ and 2 (9%) of 22 in the ACS+:HIV− group showed evidence of aspirin resistance with >20% aggregation to arachidonic acid (P = not significant [NS]). Of the patients taking clopidogrel, 1 (50%) of 2 patient in the ACS+:HIV+ group and 2 (50%) of 4 patients in the ACS+:HIV− had evidence of clopidogrel resistance with normal aggregation to ADP (P = NS). In the patients not taking aspirin or clopidogrel, the response to agonists was normal with no platelet hyperactivity in either group.

HIV Group (HIV+)

Clinical profile. The clinical and thrombotic profiles of the ACS+:HIV+ and ACS−:HIV+ groups are listed in Tables 4 and 5, respectively. The ACS+:HIV+ and ACS−:HIV+ groups were well matched with respect to age, sex, and viral load. The ACS+:HIV+ group was less immunocompromised as evidenced by higher CD4 counts (P = .01) and less patients with AIDS-defining criteria (P = .01). Of 30, 18 (60%) patients had early disease (stage A1/A2). In the ACS−:HIV+ group, 21 (70%) of 30 patients had AIDS-defining criteria.

In terms of coronary risk factors, there were more smokers in the ACS+:HIV+ group (P = .0026), and the mean HDL levels were lower compared to the ACS−:HIV+ group (P = .011).

Thrombotic profile. Thrombotic screens were performed in 21 (70%) of 30 and 29 (97%) of 30 patients in the ACS+:HIV+ and ACS−:HIV+ groups, respectively. Of 30, 1 (3%) patient in the ACS−:HIV+ group was not tested due to withdrawal of consent. Mean PC levels were lower in the ACS+:HIV+ group (P = .0163), but when analyzing the 2 groups with respect to PC deficiency (PC <70 IU/dL), there was a nonsignificant trend toward a higher degree of PC deficiency in the ACS+:HIV+ group (4 [21%] of 19 vs 4 [14%] of 29, P = .08). There were no significant differences between the groups with respect to aPL frequencies but analysis of the actual aPL titers revealed higher levels of anti-ß2-GP-1 IgM (3.0 [1.6-10.2] vs 2.1 [1.3-15.3], P = .007) in the ACS−:HIV+ group compared to the ACS+:HIV+ as well as aPT IgG (20.2 [9.3-165.1] vs 14.4 [7.5-40.2], P = .0008), aPT IgM (4.8 [1.3-9.4] vs 3.1 [1.4-30.9], P = .012), and aPT IgA (5.8 [3.3-17.3] vs 4.6 [3.0-18.2], P = .015).

Platelet studies. Light transmission aggregometry was performed in 21 (70%) of 30 patients in the ACS+:HIV+ group and 28 (93%) of 30 patients in the ACS−:HIV+ group. The findings in the ACS+:HIV+ group were described in the ACS group results section. In the ACS−:HIV+ group, 27 (95%) of 28 patients were not taking aspirin. Of these patients, 19 (70%) of 27 showed a normal aggregation response to agonists and 8 (30%) of 27 showed a ''flat'' response to arachidonic acid in the absence of antiplatelet drugs. None of the patients had evidence of platelet hyperactivity.

Discussion

Unlike other studies investigating the thrombotic profile of HIV-positive patients receiving HAART, we took the almost unique opportunity to study a HAART-naive population, thus negating the potential effects of HAART on thrombotic risk. First, when comparing the ACS+:HIV+ group and the

Table 5. HIV Group: Thrombotic Profile[a]

	ACS+:HIV+ Group	ACS−:HIV+ Group	*P* Value
Thrombotic screen at baseline, n (%)	30 (100)	30 (100)	
Hemoglobin (g/dL)	13.6 ± 2.8	12.6 ± 2.3	NS
Platelet count ($\times 10^9$/L)	301 ± 113	252 ± 83	NS
Antiphospholipid antibody frequencies, n (%)			
Anticardiolipin (IgG)	14/30 (47)	17/30 (57)	NS
Anticardiolipin (IgM)	3/30 (10)	4/30 (13)	NS
Anticardiolipin (IgA)	0/30 (0)	0/30 (0)	NS
Anti-ß-2 glycoprotein (IgG)	11/30 (37)	10/30 (33)	NS
Anti-ß-2 glycoprotein (IgM)	3/30 (10)	2/30 (7)	NS
Anti-ß-2 glycoprotein (IgA)	7/30 (23)	8/30 (27)	NS
Antiprothrombin (IgG)	26/30 (87)	29/30 (97)	NS
Antiprothrombin (IgM)	2/30 (7)	0/30 (0)	NS
Antiprothrombin (IgA)	2/30 (7)	3/30 (10)	NS
Thrombotic screen 6 weeks post event n (%)[b]	21 (70)	29 (97)	
Fibrinogen (g/L)	3.5 ± 1.0	3.6 ± 0.9	NS
D-Dimer quantitative (mg/L)	0.42 (0.2-2.49)	0.34 (0.2-5.47)	NS
Antithrombin (IU/dL)	101 ± 19	113 ± 19	NS
Protein C (IU/dL)[c]	82 ± 22	92 ± 19	.0163
Protein S (IU/dL)[c]	91 ± 29	85 ± 23	NS
APCR	2.6 ± 0.5	2.6 ± 0.4	NS
Factor 8 (IU/dL)	201 ± 87	228 ± 130	NS
VWF antigen (%)	225 ± 64	252 ± 119	NS
VWF activity (%)	108 ± 7	104 ± 9	NS
Lupus anticoagulant	0 (0)	1 (3)	NS

NOTES: ACS = acute coronary syndrome; HIV = human immunodeficiency virus; Ig = immunoglobulin; NS = not significant; VWF = von Willebrand factor.
[a] Data are presented as mean ± standard deviation, proportions, or median (min–max range).
[b] Nine patients in ACS+:HIV+ group were ineligible for testing at 6 weeks (see text). All patients in ACS−:HIV+ group were tested at baseline as this group was event free.
[c] Patients receiving warfarin were excluded from analysis (2 patients in ACS+:HIV+ group).

ACS+:HIV− group, consistent with previously published data, the HIV group was younger in age, predominantly male, with a higher percentage of cigarette smokers and lower HDL levels.[30] Active cigarette smoking is a consistent modifiable risk factor identified in many trials on HIV and arterial thrombosis,[3,31] and the high rates seen in this study are consistent with this. Besides smoking, there were less traditional risk factors for MI in the ACS+:HIV+ group, with a lower percentage of patients with hypertension, LDL hyperlipidemia, and "other coronary risk factors" compared to the HIV-negative group. The low HDL levels found in our ACS+:HIV+ group have frequently been described in treatment-naive HIV-infected individuals[32,33] and may contribute to thrombotic risk.[34] The ACS+:HIV+ group had greater evidence of thrombophilia compared to the ACS+:HIV− group. Mean PC levels were significantly lower and there was a trend toward a greater degree of PC deficiency, which would have met statistical significance if the sample size had been bigger. Despite this, we feel that the lower PC levels in the ACS+:HIV+ group are significant findings, given that "normal" values in the black South African HIV population have not been clearly defined. Along the same lines, factor VIII levels were also significantly elevated in the ACS+:HIV+ group compared to the ACS+:HIV− group, with a trend toward statistical significance when defining a cutoff value of >150 IU/dL. Antiphospholipid antibody frequencies (aCL IgG and aPT IgG) were significantly higher in the ACS+:HIV+ group. What is the significance of these findings?

Protein C deficiency is a well-known risk factor for VTE and confers an increased risk of approximately 7-fold[35] but has also been described in the pathogenesis of MI in young patients with otherwise normal coronary arteries.[36,37] Protein C deficiency results in an increased activity of factors Xa and IXa and a hypercoagulable state.[36] The mechanism of PC deficiency in HIV-positive patients is probably multifactorial, including altered synthesis and metabolism as well as low-grade disseminated intravascular coagulation (DIC) with consumptive coagulopathy.[8] Increased clotting factor VIII is known to elevate the risk of VTE,[38] but little is known about its relationship to arterial thrombosis or HIV infection. Anticardiolipin antibodies, particularly the β_2-GP-dependent variety, were strongly associated with the risk of MI in a prospective case–control analysis from the Honolulu Heart Program.[35] In a study among survivors of MI, aCLs were detected in 14% of patients compared with 3% of controls.[39] The significance of the raised aPLs in the ACS+:HIV+ group remains unclear, however, as the etiopathogenic link between aPL and thrombosis in HIV is tenuous,[14] and despite case reports, the aPLs that often present in infectious diseases are not usually associated with thrombotic complications.[40] Patients with HIV have also been shown to have higher degrees of endothelial activation, a risk factor for thrombosis, compared to the HIV-negative

population.[8,11,41] In this study, VWF antigen and activity levels were studied as a marker of endothelial activation, and although levels of VWF antigen were higher in the HIV group, this did not reach statistical significance. The incidence of aspirin resistance was similar in the 2 ACS groups and comparable with contemporary studies.[42] There was no evidence of platelet hyperactivity in either group.

Comparison of the HIV groups was performed to control for the HIV status. The patients were well matched with respect to age and sex, but the ACS+:HIV+ group was less immunocompromised, with higher CD4 counts and no evidence of opportunistic infection or AIDS-related malignancies, implying that immune dysregulation did not seem to play a significant role in the development of arterial thrombosis unlike its reported role in VTE.[43] The ACS+:HIV+ group did, however, have more classical risk factors for arterial thrombosis, with more smokers and lower HDL levels. In terms of thrombotic profiles, the ACS+:HIV+ group had significantly lower PC levels compared to the ACS−:HIV+ group despite being less immunocompromised, making the finding even more relevant as PC levels in HIV-positive patients tend to decrease with worsening immunosuppresion.[8] There were higher aPL titers (IgM anti-ß2-GP-1 and aPT [all 3 isotypes]) in the ACS−:HIV+ group compared to the ACS+:HIV+ group, possibly due to the greater degree of immunosuppresion and immune dysregulation,[40] but no difference when comparing the aPL frequencies between the groups. Several limitations of the study require comment. Although the study constitutes one of the largest prospective analyses on treatment-naive patients with ACS, the sample size in each group was relatively small, resulting in a lack of power to detect small differences between the groups, which may have been significant. In addition, not all patients underwent thrombotic screening, further weakening statistical power. Imperfect matching of the 2 HIV groups with respect to degree of immunosuppresion makes direct comparison of the thrombotic profiles difficult but the fact that the ACS–:HIV+ group had more advanced disease suggests that the degree of immunosuppresion alone is unlikely to be a significant risk factor for arterial thrombosis. Wherever possible, we have adhered to the recently published Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines in our reporting of study data.[44]

In summary, we found that treatment-naive HIV-positive patients presenting with ACS have different risk factor and thrombotic profiles compared to the HIV-negative population. Consistent with other studies, HIV-positive patients are younger, more commonly smokers, and have less traditional cardiovascular risk factors than HIV-negative patients.[30] High-density lipoprotein levels are lower in HIV-positive patients, but the significance of this with regard to arterial thrombosis is unclear and needs further investigation. Treatment-naive HIV-positive patients have lower PC levels, higher levels of factor VIII, and higher titers of aPL compared to the HIV-negative population. Furthermore, the PC levels found in the ACS+:HIV+ group were also significantly lower than that in the ACS−:HIV+ group, which constitutes an important finding in this population and warrants further investigation. In addition, further studies are required to determine the significance of the antiphospholipid antibody findings in our HIV cohort with regard to thrombotic risk. It is possible that the pathogenesis of thrombosis in these patients is multifactorial, with the interaction of conventional risk factors and HIV-specific coagulation abnormalities. To this extent, smoking is the most important modifiable risk factor and should be an important target for cardiovascular risk reduction. Thrombophilic screening in HIV-positive patients with minimal coronary risk factors presenting with ACS may be justified to aid in decision making regarding anticoagulation as a part of secondary prevention.

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CHAPTER SEVEN: ANTIPHOSPHOLIPID ANTIBODIES IN BLACK SOUTH AFRICANS WITH HIV AND ACUTE CORONARY SYNDROMES: PREVALENCE AND CLINICAL CORRELATES

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Summary: This manuscript, which has been submitted for publication, expands on Chapter 6 by exploring the role of antiphospholipid antibodies in the pathogenesis of coronary thrombosis in HIV patients, traditionally considered to be at low risk for acute coronary syndromes.

Statement of originality document: Please see Appendix.

Abstract

Background: HIV infection is associated with a high prevalence of antiphospholipid antibodies (aPL) and increased thrombotic events but the aetiopathogenic link between the two is unclear.

Methods and Results: Prospective single centre study from Soweto, South Africa, comparing the prevalence of aPL in highly active anti-retroviral therapy (HAART) naïve HIV positive and negative patients presenting with Acute Coronary Syndromes (ACS). Between March 2004 and February 2008, 30 consecutive black South African HIV patients with ACS were compared to 30 black HIV negative patients with ACS .The HIV patients were younger (43 ± 7 vs. 54 ± 13, p=0.004) and besides smoking (73% vs. 33%, p=0.002) and lower HDL levels (0.8 ± 0.3 vs. 1.1 ± 0.4, p=0.001) had fewer risk factors than the control group. HIV patients had a higher prevalence of anticardiolipin (aCL) IgG (47% vs. 10%, p=0.003) and anti-prothrombin (aPT) IgG antibodies (87% vs. 21%, p<0.001) but there was no difference in the prevalence of the antiphospholipid syndrome (44% vs. 24%, p=N/S) and aPL were not predictive of clinical or angiographic outcomes.

Conclusion: Treatment naïve black South African HIV patients with ACS are younger with fewer traditional coronary risk factors than HIV negative patients but have a higher prevalence and different expression of aPL which is likely to be an epiphenomenon of the HIV infection rather than causally linked to thrombosis and the pathogenesis of ACS.

KEYWORDS: Human Immunodeficiency Virus (HIV), Acute Coronary Syndrome (ACS), antiphospholipid antibody (aPL), antiphospholipid syndrome (APS)

Background

HIV infection is known to be associated with an increased prevalence of aPL but the link to the antiphospholipid syndrome (APS) with clinical thrombosis including myocardial infarction (MI) is tenous (1). Abuaf et al. (2) reported on the prevalence of aPL in HIV infection. Anticardiolipin antibodies (aCL) were reported to be present in 0-94%, anti-ß$_2$-glycoprotein-I (anti-ß$_2$-GPI) in 4-47%, anti-prothrombin (aPT) in 2-12% of patients with lupus anticoagulant (LA) found in 0-53.5%. Very few data exist on the prevalence of aPL in black patients especially aPT and IgA aPL isotypes in the setting of infections including HIV (3). Loizou et al.(3) reported on the prevalence of aPL in 100 black South African HIV positive patients. There was a low prevalence of anti-ß$_2$-GPI (6%), all exclusively belonging to the IgA isotype, as well as aCL (7%), which were mainly positive for IgG. A prevalence of 43% (mainly IgG) aPT was found showing that the pattern of aPL in black South Africans differs from that found in caucasians (3). Despite the unclear association between HIV, aPL and MI, there have been case reports in the non-HIV setting suggesting an association. A prospective case control analysis from the Honolulu Heart Program found that anticardiolipin antibodies, particularly the ß$_2$-glycoprotein-dependent variety were strongly associated with the risk of MI (4). In a study among survivors of MI, anticardiolipin antibodies were detected in 14% of patients compared with 3% of controls (5). The antiphospholipid syndrome is found more commonly in populations with MI who have a low burden of conventional cardiovascular risk factors with little or no evidence of atherosclerotic disease and the condition needs to be considered in patients with normal appearing infarct related arteries (4). We have shown that treatment-naïve HIV positive black South African patients presenting with acute coronary syndromes (ACS) are younger with fewer traditional risk factors

compared to HIV negative patients and have less atherosclerotic burden but higher thrombotic burden on angiography (6). In a subsequent study we showed that this group of HIV patients have evidence of thrombophilia as evidenced by lower protein C and higher factor VIII levels (7). In addition, preliminary results showed a higher prevalence of aPL the significance of which is uncertain.

Study Aims

It is within this context, that we investigated the role of aPL as a risk factor for ACS in treatment-naïve black South Africans with minimal traditional risk factors. We hypothesized that HIV patients with ACS, compared to HIV negative patients, would have a higher prevalence of aPL with a higher prevalence of the antiphospholipid syndrome (APS) and that aPL would be causally related to thrombosis and ACS.

Methods

Study design and patient enrollment

We conducted a prospective single centre study in the Department of Cardiology at Chris Hani Baragwanth Hospital, Soweto, South Africa. The protocol was approved by the ethics committee of the University of the Witwatersrand and adheres to the Declaration of Helsinki. All patients gave informed consent before study entry. Between March 2004 and February 2008, 30 consecutive black HIV patients presenting with ACS **(ACS+ : HIV+ group)** were enrolled. For each HIV patient with ACS we selected the first presenting non-HIV black patient with ACS as a case-control comparator (**ACS+ : HIV- group**). In addition a second control group consisting of 30 asymptomatic HIV patients matched for age, sex and ethnicity **(ACS- : HIV+ group)** were recruited from the HIV

clinic. ACS was defined as either ST-elevation myocardial infarction, non ST-elevation myocardial infarction or unstable angina. Patients were categorized as having diabetes, hypertension or dyslipidemia when being treated chronically for these conditions or when diagnosed with the condition on admission. Patients were classified as having "other" coronary risk factors if any of the following conditions were present: i) Family history of premature CAD (men <55 yrs, women <65 yrs), ii) chronic kidney disease , iii) post menopausal state and iv) abdominal obesity (abdominal circumference > 102cm in men and 88cm in women) . Demographic data was recorded for each patient and anthropometric measurements including weight, height, body mass index (BMI) , waist to hip ratio and abdominal circumference (AC) were measured on admission according to guidelines set out in the Interheart study (8). Infection with HIV was diagnosed with a standard enzyme linked immunosorbent assay and Western blot techniques after obtaining consent and offering pretest counseling. In the HIV group, Plasma HIV RNA level was determined by quantitative polymerase chain reaction. CD4 count was determined by flow cytometry and patients were staged according to the CDC staging system (9). Patients with ACS were managed according to accepted guidelines set out by the European Heart Association (10, 11) and followed up at the Chris Hani Baragwanath cardiac clinic.

Laboratory methods

Blood was obtained by clean venipuncture with seated subjects and non-fasting venous blood was drawn into plastic tubes and allowed to clot at 37 degrees and then cetrifuged at 2500xg for 8 minutes for sera preparation. The serum was immediately stored at -80 degrees until use. All sera were thawed only once in a water bath at 37 degrees Celcius for 15 minutes before testing. The analysis of aPL was performed at Lancet Laboratories, Johannesburg. Antiphospholipid antibodies including aCL, anti- ß$_2$-GP-1, and aPT (IgG, IgM, IgA) were measured at baseline in all patients and at least 12 weeks later in the ACS groups using commercial enzyme linked immunosorbent assay (ELISA) kits (Orgentec Diagnostika, Mainz,Germany). The results were expressed in units, according to the manufacturer's instructions. IgG, IgM and IgA aCL were expressed as U/mL. To establish normal values of aPL in our population we used the blood samples of 100 asymptomatic HIV negative black South African blood donors matched for age and sex to the study population. Results for each of the aPL measured were considered positive when the optical density obtained for each patient exceeded that of the mean value plus 2 standard deviations (SD) of the 100 sera from black South African normal healthy subjects. The antiphospholipid syndrome (APS) was diagnosed in patients with ACS who had the presence of aPL (aCL IgG/ IgM and/or anti- ß$_2$-GP-1 IgG/ IgM) in titres greater than the mean plus 2SD from normal healthy subjects on two occasions at least 12 weeks apart (12).

Stastistical analysis

Statistical analysis was performed using SAS 9.1 software (SAS, Cary, NC, USA). Normally distributed continuous data are presented as the mean (± standard deviation), and variables with non-Gaussian distribution as the median (min-max range). Categorical data are presented as frequencies and percentages. The initial analysis compared variables between the 3 groups using the one way anova test for continuous variables with normal distribution and the Kruskal Wallis test in case of non-normal distribution. For categorical variables the Chi square test was performed with a Fisher exact test when necessary. Significant differences between variables in the 3 groups was assumed at $p<0.05$. Subgroup analysis with multiple pairwise comparisons was then performed applying the Bonferroni correction with a p value< 0.0166 considered significant. Univariate logistic regression was performed to determine predictors of the variables: aCL IgG, aPT IgG and APS: data presented as odds ratios (OR) with 95% confidence intervals (CI).

Results

Acute Coronary Syndrome (ACS+) group

The clinical profile and prevalence of aPL in the ACS+ : HIV+ and ACS+ : HIV-groups are listed in **Table 1.** HIV positive patients were younger with a similar sex distribution. Besides smoking, (33% vs. 73%, p=0.004), coronary risk factors were higher in the ACS+ : HIV- group with more hypertension (p=0.0001), LDL hyperlipidaemia (p=0.003), diabetes mellitus (p=0.03) and "other coronary risk factors" (p=0.0001). The ACS+ : HIV+ group had lower HDL levels (p=0.0006) and a lower mean BMI (p=0.008). The ACS+: HIV+ group had higher frequencies of aCL IgG (47% vs. 10%, p=0.003) and aPT IgG (87% vs. 21%, p<0.001) compared to the ACS+: HIV- group. There were no differences between groups with respect to the other aPL frequencies. The ACS group (60 patients) was then analysed to determine the prevalence of APS. In the ACS+ : HIV+ group, 18/30(60%) patients had aPL results from two separate occasions, 12 weeks apart as per diagnostic guidelines (12). 12/30(40%) did not have repeat testing owing to 9/30(30%) deaths and 3/30(10%) patients lost to follow up. In the ACS+: HIV- group, 25/29(86%) patients had aPL results from two separate occasions, 12 weeks apart with 4/29(14%) patients dying prior to repeat testing. The diagnostic criteria for APS were met in 8/18(44%) HIV patients vs. 6/25(24%) HIV negative patients, p=0.28. In the ACS+: HIV+ group, 7/8(88%) had aCL IgG antibodies, 1/8(13%) aCL IgM, 6/8(75%) anti- $ß_2$-GP-1 IgG and 1/8(13%) anti- $ß_2$-GP-1IgM. 4/8(50%) patients had the presence of both aCL IgG and anti- $ß_2$-GP-1IgG antibodies. In the ACS+ : HIV- group, 3/6(50%) had aCL

IgG antibodies, 1/6(17%) aCL IgM and 4/6(67%) anti- ß$_2$-GP-1IgG. 2/6(33%) had the presence of both aCL IgG and anti- ß$_2$-GP-1IgG antibodies.

HIV (HIV+) group

The clinical profile and prevalence of aPL in the ACS+ : HIV+ and ACS- : HIV+ groups are listed in **Table 2.**

The ACS+ : HIV+ and ACS- : HIV+ groups were well matched with respect to age, sex and viral load. The ACS+ : HIV+ group were less immunocompromised as evidenced by higher CD4 counts (p=0.013) and less patients with AIDS defining criteria (p=0.01) which were all based on a CD4 count<200 cells/ml^3. There were no opportunistic infections or HIV related malignancies in the ACS+ : HIV+ group and 18/30(60%) patients had early disease being classified as either stage A1 or A2 (9). In the ACS- : HIV+ group, 21/30(70%) patients had AIDS, 18/30(60%) due to a CD4 count <200 cells/ml^3 and 3/30(10%) patients with either oppurtunistic infections or AIDS related malignancies: 1/30(3%) patient having active pulmonary tuberculosis, 1/30(30%) with cutaneous Kaposi's Sarcoma and 1/30(3%) with a non-Hodgkins lymphoma. 6/30(20%) had early disease (stage A1 or A2). In terms of coronary risk factors, there were more smokers in the ACS+ : HIV+ group (p=0.0026) and the mean HDL levels were lower (p=0.011) compared to the ACS- : HIV+ group. There were no significant differences between the groups with respect to aPL frequencies but analysis of the actual aPL titres revealed higher levels of ß$_2$-GP-1 IgM [3.0(1.6-10.2) vs. 2.1(1.3-15.3), p=0.007] in the ACS- : HIV+ group compared to the ACS+ : HIV+ as well as aPT IgG [20.2(9.3-165.1) vs. 14.4(7.5-40.2), p=0.0008], aPT IgM [4.8(1.3-9.4) vs. 3.1(1.4-30.9), p=0.012] and aPT IgA [5.8(3.3-17.3) vs. 4.6(3.0-18.2), p=0.015]. On univariate logistic regression, HIV

infection was predictive of the presence of aCL IgG (OR 2.94, CI 1.60-5.40, p=0.0005) and aPT IgG antibodies (OR 16.07, CI 5.38-49.94, p<0.0001) but did not predict APS (OR 2.53, CI 0.69-9.36, p=0.16). The presence of aPL's (aCL IgG , aPT IgG) and APS did not predict any of the clinical or angiographic outcomes in the study including markers of atherosclerotic and thrombotic burden.

Discussion

HIV infection is known to be associated with increased frequencies of aPL (13) and the antiphospholipid syndrome is found more commonly in populations with ACS who have a low burden of conventional cardiovascular risk factors with little or no evidence of atherosclerotic disease (4) such as our HIV cohort. No data currently exists, however, on aPL frequencies and clinical correlates in HAART-naïve HIV patients. We took the almost unique opportunity to study a HAART-naïve population thus negating the effects of HAART on thrombotic risk. Firstly, when comparing the ACS+: HIV+ group and the ACS+: HIV- group, consistent with previously published data, the HIV group were younger in age, predominantly male, with a higher percentage of cigarette smokers and lower HDL levels (14). Besides smoking, there were less traditional risk factors for ACS in the ACS+: HIV+ group. With respect to aPL frequencies in the ACS group, significant differences were found with HIV patients having higher frequencies of aCL IgG and aPT IgG antibodies. The incidence of APS was, however, similar in both groups. What is the significance of these findings? The exact role of aPL in the aetiopathogenesis of thrombosis in HIV infected patients is controversial. Despite the findings of high aPL frequencies (2) and case reports of APS in HIV patients, antiphospholipid antibodies

often present in infectious diseases are not usually associated with thrombotic complications (3). In order to address the question of significance of the aPL findings in our HIV patients with ACS, we looked at a similar HIV group matched for age, sex and degree of immunosuppression (ACS- : HIV+ group). The ACS+: HIV+ group, had a different pattern of aPL expression compared to recent studies investigating a predominantly caucasian population which reported aCL to be positive in 36-88%, anti-ß$_2$-GP-1 in 4-27%, and aPT in 2-12% of patients (13, 15-17). In keeping with the study by Loizou et al (3) we found a high prevalence of aPT IgG antibodies in our black HIV cohort with 87% in the ACS+ : HIV+ group and 97% in the ACS- : HIV+ group. The rates of aCL and anti- ß$_2$-GP-1 antibodies seen in our black HIV population were similar to those described in the recent studies on Caucasian patients (13, 15-17). Comparing the aPL frequencies within the HIV group, there were no statistically significant differences between the ACS+ : HIV+ and ACS- : HIV+ groups but analysis of the actual aPL titres revealed higher levels of ß$_2$-GP-1 IgM as well as aPT (IgG,IgM,IgA) in the ACS- : HIV+ group possibly due to a greater degree of immunosuppresion and immune dysregulation (3), suggesting that the higher frequencies of aPL seen in HIV patients are an epiphenomenon of HIV infection rather than causally linked to thrombotic events.The aPL seen in HIV patients could be induced by disturbances in regulation of cellular and humoral immunity, as a secondary consequence of HIV infection. Alternatively, their induction might result from the exposure of cell wall phospholipids as a consequence of damaged body cells resulting from the HIV-related inflammatory milieu (3). Several limitations of the study require comment. Although the study constitutes one of the largest prospective analyses on treatment-naïve patients with ACS, the sample size in

each group was relatively small resulting in a lack of power to detect small differences between the groups which may have been significant. In addition, not all patients had repeat testing at 12 weeks which would have influenced the reported incidence of APS in the patients who had thrombotic events. Wherever possible, however, we have adhered to the recently published STROBE guidelines in our reporting of study data (18).

In summary, we found that treatment-naïve HIV patients presenting with ACS have different risk factors and clinical features compared to the HIV negative population as well as a higher prevalence and different pattern of aPL expression. It is unlikely that aPL in this group of patients are causally linked to ACS and are more likely an epiphenomenon of HIV infection. It is possible that the pathogenesis of thrombosis in these patients is multifactorial with the interaction of conventional risk factors and other HIV-specific coagulation abnormalities which warrants further study. To this extent smoking is an important modifiable risk factor and should be an important target for cardiovascular risk reduction. Routine screening for antiphospholipid antibodies in HIV patients presenting with ACS and minimal risk factors cannot be justified based on these findings.

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Disclosures

No conflict of interest noted

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Table 1 ACS Group: Clinical profile and aPL prevalence

	ACS+ : HIV+	ACS+ : HIV-	p value
Demographic Profile:			
Black African n(%)	30(100)	30(100)	1.0
Mean Age (years)	43 ± 7	54 ± 13	<0.001
Men (%)	20(67)	18(60)	N/S
Coronary risk factors n(%):			
Smoking	22(73)	10(33)	0.004
Diabetes Mellitus	1(3)	7(23)	0.05
Hypertension	7(23)	23(77)	<0.001
Total cholesterol (mmol/l)	3.6 ± 1.0	4.6 ± 1.4	0.003
LDL cholesterol (mmol/l)	2.2 ± 0.9	3.0 ± 1.2	0.003
HDL cholesterol (mmol/l)	0.8 ± 0.3	1.1 ± 0.4	<0.001
Triglycerides (mmol/l)	1.4 ± 0.8	1.1+/-0.4	N/S
BMI (kg/m^2)	25 ± 5	28 ± 5	0.008
Other coronary risk factors	2(7)	16(53)	<0.001
Baseline aPL frequencies(%):			
Blood samples n(%)	30(100)	29(97)*	N/S
Anticardiolipin (IgG)	14(47)	3(10)	0.003
Anticardiolipin (IgM)	3(10)	1(3)	N/S
Anticardiolipin (IgA)	0(0)	3(10)	N/S
Beta-2 Glycoprotein (IgG)	11(37)	6(21)	N/S
Beta-2 Glycoprotein (IgM)	3(10)	0(0)	N/S
Beta-2 Glycoprotein (IgA)	7(23)	12(41)	N/S
Anti-Prothrombin (IgG)	26(87)	6(21)	<0.001
Anti-Prothrombin (IgM)	2(7)	0(0)	N/S
Anti-Prothrombin (IgA)	2(7)	1(3)	N/S
Antiphospholipid syndrome (%):	8/18(44)	6/25(24)	N/S

Legend: Data are presented as the mean ± standard deviation or proportions

Key: BMI= body mass index
aPL= antiphospholipid antibody

* 1 patient not analysed due to an inadequate blood sample

Table 2 HIV group: Clinical profile and aPL prevalence

	ACS+ : HIV+	ACS- : HIV+	p value
Demographic Profile:			
Black African n(%)	30(100)	30(100)	1.0
Mean Age (years)	43 ± 7	41 ± 8	N/S
Men (%)	20(67)	19(63)	N/S
HIV related factors:			
CD4(cells/mm^3) median(range)	230 (30 -1356)	125 (6-1041)	0.013
Viral load (RNA copies/ml) median(range)	29000 (25 - $7x10^5$)	54000(25-11x105)	N/S
AIDS defining criteria n(%)	11(37)	21(70)	0.01
Current oppurtunistic infection	0	1(3)	N/S
HIV related malignancies	0	2(7)	N/S
Coronary risk factors n(%):			
Smoking	22(73)	11(37)	0.003
Diabetes Mellitus	1(3)	0(0)	N/S
Hypertension	7(23)	2(7)	N/S
Total cholesterol (mmol/l)	3.6 ± 1.0	3.7+/-0.8	N/S
LDL cholesterol (mmol/l)	2.2 ± 0.9	2.0 ± 0.5	N/S
HDL cholesterol (mmol/l)	0.8 ± 0.3	1.0 ± 0.5	0.011
Triglycerides (mmol/l)	1.4 ± 0.8	1.4+/-0.8	N/S
BMI (kg/m^2)	25 ± 5	21± 4	0.003
Other coronary risk factors	2(7)	0(0)	N/S
Baseline aPL frequencies (%):			
Blood samples n(%)	30(100)	30(100)	1.0
Anticardiolipin (IgG)	14/30(47)	17/30(57)	N/S
Anticardiolipin (IgM)	3/30(10)	4/30(13)	N/S
Anticardiolipin (IgA)	0/30(0)	0/30(0)	N/S
Beta-2 Glycoprotein (IgG)	11/30(37)	10/30(33)	N/S
Beta-2 Glycoprotein (IgM)	3/30(10)	2/30(7)	N/S
Beta-2 Glycoprotein (IgA)	7/30(23)	8/30(27)	N/S
Anti-Prothrombin (IgG)	26/30(87)	29/30(97)	N/S
Anti-Prothrombin (IgM)	2/30(7)	0/30(0)	N/S
Anti-Prothrombin (IgA)	2/30(7)	3/30(10)	N/S

Legend: Data are presented as the mean ± standard deviation or proportions

Key: BMI= body mass index
aPL= antiphospholipid antibody

CHAPTER EIGHT: MARKERS OF INFLAMMATION AND ENDOTHELIAL ACTIVATION IN BLACK SOUTH AFRICANS WITH HIV AND ACUTE CORONARY SYNDROMES

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Summary: This manuscript, submitted for publication, explores the possible contribution of inflammation and endothelial activation in the pathogenesis of thrombosis and proposes a mechanistic link between the two in treatment-naïve HIV positive patients.

Statement of originality document: Please see Appendix.

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Full Length Research Paper

Markers of inflammation and endothelial activation in black South Africans with HIV and acute coronary syndromes

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HIV infection is associated with a pro-inflammatory and thrombophilic state but little is known about the link between inflammation and thrombosis in treatment-naïve patients with acute coronary syndromes (ACS). Prospective single centre study was conducted in Soweto, South Africa, comparing markers of inflammation and endothelial cell activation in highly active anti-retroviral therapy-naïve HIV positive and negative patients presenting with ACS. Between March 2004 and February 2008, 30 consecutive black South African HIV patients with ACS were compared to 30 black HIV negative patients with ACS. The HIV patients were younger (43 ± 7 vs. 54 ± 13, p = 0.004) and besides smoking (73% vs. 33%, p = 0.002) and lower HDL levels (0.8 ± 0.3 vs. 1.1 ± 0.4, p = 0.001) had fewer risk factors than the control group. At baseline, HIV patients had higher levels of tumour necrosis factor-α [5.8 (1.7 - 15.0) vs. 0.19 (0.19 - 19.8) ng/ml, p = 0.0004] and vascular cell adhesion molecule-1 [263.3 (0.38 - 778.5) vs. 151.3 (80.6 - 416.3) ng/ml, p = 0.007] compared to HIV negative patients as well as higher levels of macrophage chemoattractant protein-1 at six months [70 (30 -130) vs. 50 (30 - 90) ng/L, p = 0.004]. Treatment-naïve black South African patients with HIV and ACS have evidence of a pro-inflammatory state and greater degree of endothelial cell activation compared to HIV negative patients, both of which may play a direct role in the pathogenesis of ACS in this otherwise low risk population. MCP-1 may play an important role in HIV-associated coronary artery disease.

Key words: Human immunodeficiency virus (HIV), acute coronary syndrome (ACS), inflammation, endothelial dysfunction, thrombosis.

INTRODUCTION

Inflammation and endothelial cell activation are key components in the initiation, progression and thrombotic complications of atherosclerotic coronary artery disease (CAD) (Koenig and Khuseyinova, 2007). C reactive protein (CRP) and Interleukin-6 (IL-6), both pro-inflammatory cytokines, as well as having pathogenic roles in atherosclerosis, also have strong and independent prognostic implications in patients with atherosclerotic vascular disease and acute coronary syndromes (ACS) (Koenig and Khuseyinova, 2007). Monocyte chemoattractant protein-1 (MCP-1) is a potent activator of macrophages and monocytes and is actively involved in the initiation and progression of atherosclerosis (de Lemos et al., 2003). A significant association between increasing concentrations of the endothelial activation markers [soluble E-selectin (sE-selectin), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cellular adhesion molecule-1 (sVCAM-1)] and future cardiac events was shown in apparently healthy individuals in the ARIC (atherosclerosis risk in communities) study (Hwang et al., 1997). HIV infection is characterised by a profound inflammatory response with elevated levels of a number of pro-inflammatory

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cytokines including tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6 and CRP (Hsue et al., 2004; Hazenberg et al., 2000; Frostegard et al., 1999) which persist even after the introduction of highly active anti-retroviral therapy (HAART) (Valdez et al., 2002) and contribute to endothelial dysfunction (Gupta et al., 2008), atherogenesis and a prothrombotic state. Among HIV patients with subclinical atherosclerosis by carotid and femoral ultrasound, MCP-1 plasma levels were higher compared to HIV patients without atherosclerosis (Alonso-Villaverde et al., 2004). Endothelial activation markers including sE-selectin, sICAM-1 and sVCAM-1 have all been shown to be elevated in HIV positive patients (de Gaetano et al., 2004) but the link between *in vitro* findings and clinical events in HIV patients is still lacking. HIV-associated coronary artery disease described in the developed world is characterised by accelerated atherosclerosis and is thought to be due to a synergistic effect of viral mediated effects and the atherogenic effects of protease inhibitor (PI) containing HAART (Hsue and Waters, 2005). Little data exists on the nature of HIV-associated CAD in the developing world and specifically in South Africa where risk factors for CAD have traditionally been low (di Bisceglie et al., 1982). Within the above context, we have shown that treatment-naïve HIV positive black South Africans presenting with ACS are younger with fewer traditional risk factors compared to HIV negative patients with less atherosclerotic burden but a higher thrombotic burden on angiography (Becker et al., 2010). In a subsequent study we showed that this group of HIV patients had evidence of thrombophilia as evidenced by lower protein C and higher Factor VIII levels (Becker et al., 2010). We sought to determine whether markers of inflammation and endothelial cell activation differ between treatment-naïve black HIV positive patients with ACS compared to the HIV negative population and in so doing try and determine their importance in the pathogenesis of HIV-associated CAD and ACS. We hypothesized that HIV patients would have a higher inflammatory burden and higher levels of endothelial cell activation markers and that these would be contributory in the pathogenesis of ACS.

METHODS

Study design and patient enrollment

We conducted a prospective single centre study in the Department of Cardiology at Chris Hani Baragwanth Hospital, Soweto, South Africa. The protocol was approved by the ethics committee of the University of the Witwatersrand and adheres to the Declaration of Helsinki. All patients gave informed consent before study entry. Between March 2004 and February 2008, 30 consecutive HIV patients presenting with ACS (ACS+/ HIV+ group) were enrolled. For each HIV patient with ACS, we selected the first presenting non-HIV patient with ACS as a case-control comparator (ACS+/ HIV- group). In addition, a second control group without ACS, consisting of 30 asymptomatic HIV patients matched for age, sex and ethnicity (HIV+ alone group) were recruited from the HIV clinic. Consistent with current guidelines (Van der Werf, 2003; Bertrand et al., 2002), ACS was defined as either ST-elevation myocardial infarction, non ST-elevation myocardial infarction or unstable angina. Patients were categorized as having diabetes, hypertension or dyslipidemia when being treated chronically for these conditions or when diagnosed with the condition on admission. Patients were classified as having “other” coronary risk factors if any of the following conditions were present: (1) Family history of premature CAD (men < 55 years, women < 65 years); (2) chronic kidney disease; (3) post menopausal state and (4) abdominal obesity (abdominal circumference > 102 cm in men and 88 cm in women). Demographic data was recorded for each patient and anthropometric measurements including weight, height, body mass index (BMI) , waist to hip ratio and abdominal circumference (AC) were measured on admission according to guidelines set out in the INTERHEART study (Yusuf, 2004). Infection with HIV was diagnosed with a standard enzyme linked immunosorbent assay and Western blot techniques after obtaining consent and offering pretest counseling. In the HIV group, Plasma HIV RNA level was determined by quantitative polymerase chain reaction. CD4 count was determined by flow cytometry and patients were staged according to the CDC staging system (CDC, 1993).

Laboratory methods

Standardised venipuncture was used to collect blood samples (20 ml in an EDTA tube) in all patients on enrollment and again at 6 months in the two ACS groups only. Plasma was separated by centrifugation at 2500 rpm for 12 min within 15 min of collection. Aliquots were stored at -70°C. All plasma samples used in these studies were thawed only once for analysis. Markers selected for analysis included TNF-α, IL-6, high sensitivity-CRP (hs-CRP) [pro-inflammatory cytokines], MCP-1 [chemokine] and sE-selectin, sICAM-1 and sVCAM-1 [endothelial CAMS (cellular adhesion molecules)]. TNF-α, IL-6, MCP-1, sICAM-1 and sVCAM-1 were measured using a Bio-Plex cytokine assay (Bio-Rad Laboratories, Hercules, CA).hs-CRP was measured by latex immunoassay (CRP Vario, Sentinal Diagnostics, Milano, Italy) and E-selectin with an enzyme-linked immunosorbent assay (Human sE-selectin immunoassay kit, Invitrogen Corporation, Carlsbad, CA).

Stastistical analysis

Statistical analysis was performed using SAS 9.1 software (SAS, Cary, NC, USA). Normally distributed continuous data are presented as the mean (± standard deviation), and variables with non-Gaussian distribution as the median (min - max range). Categorical data are presented as frequencies and percentages. The initial analysis compared variables between the 3 groups using the one way anova test for continuous variables with normal distribution and the Kruskal Wallis test in case of non-normal distribution. For categorical variables the Chi-square test was performed with a Fisher exact test when necessary. Significant differences between variables in the 3 groups was assumed at $p < 0.05$. Subgroup analysis with multiple pair-wise comparisons was then performed applying the Bonferroni correction with a p value < 0.0166 considered significant.

RESULTS

The clinical characteristics of the three study groups are presented in Table 1 and markers of inflammation and endothelial activation in Table 2.

Table 1 Clinical characteristics.

	ACS+ / HIV+ (n = 30)	ACS+ /HIV- (n = 30)	HIV+ alone (n = 30)	p value
Demographic Profile				
Black African n (%)	30 (100)	30 (100)	30 (100)	-
Mean age (years)	43 ± 7	54 ± 13*	41 ± 8	0.0002
Men (%)	20(67)	18(60)	19(63)	N/S
Coronary risk factors n (%)				
Smoking	22(73)	10(33)*	11(37)*	0.004;0.003
Diabetes Mellitus	1(3)	7(23)*	0(0)	0.05
Hypertension	7(23)	23(77)*	2(7)	< 0.001
Total cholesterol (mmol/l)	3.6 ± 1.0	4.6 ± 1.4*	3.7+/-0.8	0.003
LDL cholesterol (mmol/l)	2.2 ± 0.9	3.0 ± 1.2*	2.0 ± 0.5	0.003
HDL cholesterol (mmol/l)	0.8 ± 0.3	1.1 ± 0.4*	1.0 ± 0.5*	< 0.001; 0.011
Triglycerides (mmol/l)	1.4 ± 0.8	1.1+/-0.4	1.4+/-0.8	N/S
BMI (kg/m^2)	25 ± 5	28 ± 5*	21± 4*	0.008;0.003
Other coronary risk factors	2 (7)	16 (53)*	0 (0)	< 0.001
HIV related factors				
CD4 (cells/mm^3) median (range)	230 (30 - 1356)	N/A	125 (6 - 1041)	0.013
Viral load (RNA copies/ml) median (range)	29000 (25 – 7 x 10^5)	N/A	54000 (25 – 11 x 10^5)	N/S
AIDS defining criteria n (%)	11 (37)	N/A	21 (70)	0.01
Current opportunistic infection	0	N/A	1 (3)	N/S
HIV related malignancies	0	N/A	2 (7)	N/S

Data are presented as mean ± standard deviation, percentages or median (range)
Key: BMI = Body mass index; * p < 0.05 vs. ACS+/ HIV+ group.

Patients presenting with an acute coronary syndrome

Patients in the ACS+/ HIV+ group were younger with a similar sex distribution. Besides smoking, (33% vs. 73%, p = 0.004), coronary risk factors were higher in the ACS+/ HIV- group with more hypertension (p = 0.0001), LDL hyperlipidaemia (p = 0.003), diabetes mellitus (p = 0.03) and "other coronary risk factors" (p = 0.0001). Alternatively, this group had lower HDL levels (p = 0.0006) and a lower mean BMI (p = 0.008). The angiographic features of this group have been described previously (Becker et al., 2010).

At baseline, the ACS+/ HIV+ group had higher levels of TNF-α [5.8 (1.7 - 15.0) vs. 0.19 (0.19 - 19.8) ng/ml, p = 0.0004] but there were no differences between the levels of IL-6, MCP-1 or hs-CRP. This group did however have higher levels of VCAM-1 [263.3 (0.38 - 778.5) vs. 151.3 (80.6 - 416.3) ng/ml, p = 0.007] but there was no difference in levels of ICAM-1 or E-selectin. A 6 month comparison was made in this group to correct for the influence of acute thrombosis on inflammatory and endothelial markers. At 6 months, 12/30 (40%) patients in the ACS+/ HIV+ and 3/30 (10%) in the ACS+/ HIV- groups had died, and therefore did not have repeat testing. MCP-1 levels were higher in the ACS+/HIV+ group [70 (30 - 130) vs. 50 (30 - 90) ng/L, p = 0.004]. Alternatively, ICAM-1 levels were significantly higher in the ACS+/HIV- group [273.6 (64.8 - 1128.7) vs. 94.5 (18.8 - 245.3) ng/ml, p < 0.0001] as were levels of E-selectin [31.6 (2.4 - 80.4) vs. 8.8 (2.4 - 54.1) ng/ml, p = 0.004].

HIV+ alone patients

The ACS+/ HIV+ and HIV+ alone groups were well matched with respect to age, sex and viral load. Patients with an ACS were less immune compromised as evidenced by higher CD4 counts (p = 0.013) and less patients with AIDS defining criteria (p = 0.01) which were all based on a CD4 count< 200 cells/ml^3. There were no opportunistic infections or HIV related malignancies in the ACS+/ HIV+ group and 18/30 (60%) patients had early disease being classified as either stage A1 or A2 (CDC, 1993). In the HIV+ alone group, 21/30 (70%) patients had AIDS, 18/30 (60%) due to a CD4 count < 200 cells/ml^3 and 3/30 (10%) patients with either opportunistic infections or AIDS related malignancies. 6/30 (20%) had

Table 2. Markers of Inflammation and endothelial activation.

	ACS+/ HIV+ (n = 30)	ACS+/ HIV- (n = 30)	HIV+ alone (n = 30)	p value
Baseline Inflammatory markers				
TNF-α (ng/ml)	5.8(1.7 - 15.0)	0.19 (0.19 - 19.8)*	3.5(0.38 - 94.1)	0.0004
IL-6 (ng/ml)	26.2(3.3 - 479.0)	23.2(0.92 - 277.4)	6.2(1.5 - 21.7)*	< 0.0001
MCP-1(ng/L)	60(20 - 720)	50(30 - 90)	60(20 - 510)	N/S
hs-CRP (mg/L)	36.1(0.5 - 173.8)	59.9(2.3 - 255.1)	6.1(0.34 - 51.7)*	< 0.0001
Baseline endothelial activation markers				
ICAM-1(ng/ml)	81.3(0.48 - 179.9)	112.3(41.2 - 248.4)	350.4(208.1 - 1659.5)*	< 0.0001
VCAM-1 (ng/ml)	263.3(0.38 - 778.5)	151.3(80.6 - 416.3)*	395.0(29.9 - 1141.9)*	0.007;0.0016
E-selectin (ng/ml)	18.5(2.4 - 65.9)	21.3(2.4 - 58.2)	44.1(2.4 - 148.2)*	< 0.0001
6 month Inflammatory markers				
TNF-α (ng/ml)	3.1(0.19 - 8.2)	4.3(0.19 - 15.3)	N/A	N/S
IL-6 (ng/ml)	5.6(0.92 - 54.1)	5.9(0.92 - 24.2)	N/A	N/S
MCP-1(ng/L)	70(30 - 130)	50(30 - 90)*	N/A	0.004
hs-CRP (mg/L)	4.5(1.4 - 109.8)	4.3 (1.1 - 71.7)	N/A	N/S
6 month endothelial activation markers				
ICAM-1(ng/ml)	94.5(18.8 - 245.3)	273.6(64.8 - 1128.7)*	N/A	< 0.0001
VCAM-1(ng/ml)	296.1(153.0 - 555.5)	251.5(120.6 - 613.4)	N/A	N/S
E-selectin (ng/ml)	8.8(2.4 - 54.1)	31.6(2.4 - 80.4)*	N/A	0.004

Data are presented as median (min - max range)
Key: TNF-α, Tumour necrosis factor- α; IL-6, Interleukin-6; MCP-1, Macrophage chemoattractant protein-1; hs-CRP, high sensitivity C reactive protein; ICAM-1, Intercellular adhesion molecule-1; VCAM-1, Vascular cellular adhesion molecule-1; sE-selectin, soluble Endothelial-selectin; * $p < 0.05$ vs. ACS+/ HIV+ group.

early disease (stage A1 or A2). In terms of coronary risk factors, there were more smokers in the ACS+/HIV+ group ($p = 0.0026$) and the mean HDL levels were lower ($p = 0.011$) compared to the HIV+ alone group. At baseline, markers of inflammation were significantly higher in the HIV+/ACS+ group with higher levels of IL-6 [26.2 (3.3 - 479) vs. 6.2 (1.5 - 21.7) ng/ml, p,0.0001] and hs-CRP [36.1 (0.5 - 173.8) vs. 6.1 (0.34 - 51.7) mg/L, p, 0.0001]. Levels of endothelial activation markers were all higher in the HIV+ alone group: ICAM-1 [350.4 (208.1 - 1659.5) vs. 81.3 (0.48 - 179.9) ng/ml, p, 0.0001], VCAM-1 [395 (29.9 - 1141.9) vs. 263.3 (0.38 - 778.5) ng/ml, $p = 0.0016$] and E-selectin [44.1 (2.4 - 148.2) vs. 18.5 (2.4 - 65.9) ng/ml, $p < 0.0001$].

DISCUSSION

By studying a treatment naïve group of HIV patients with ACS we were able to look at markers of inflammation and endothelial cell activation without having the confounding effects of HAART. Considering the ACS+ group first, the pro-inflammatory cytokine TNF-α was significantly higher, at baseline, in HIV patients (ACS+/HIV+ group) compared to their HIV negative counterparts (ACS+/HIV- group) despite being younger with less traditional risk factors for CAD. There was no evidence of infectious disease in either group at the time of blood collection making it unlikely that the difference in inflammatory markers could be explained by an underlying, confounding infectious process which has been shown previously to be a precipitant in the pathogenesis of ACS (Ross, 1999). Baseline markers of endothelial activation were also different between the two groups with higher levels of VCAM-1 in the HIV group. VCAM-1 is a member of the immunoglobulin super-family and is involved in the firm adhesion and transmigration of leucocytes through endothelial cells (de Gaetano et al., 2004). Increased levels of VCAM-1 have been shown to occur with stimulation of endothelial cells with pro-inflammatory cytokines (Figure 1) and have a strong predictive value of future cardiovascular events (Blankenberg et al., 2003). This pro-inflammatory state is likely related to the well described effects of the HIV virus on CD4 cell and macrophage activation with consequent elaboration of pro-inflammatory cytokines including TNF-α and the interleukins (Frostegard et al., 1999) which in turn may lead to accelerated atherosclerosis and a prothrombotic

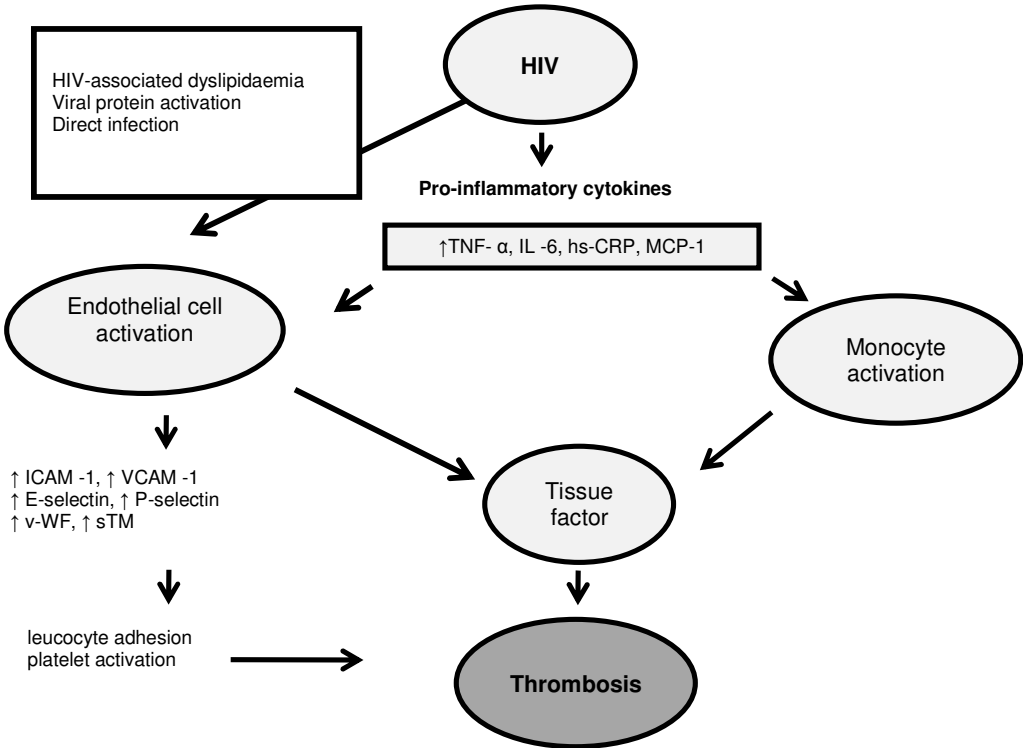


Figure 1. A proposed link between HIV, inflammation and thrombosis.
Key: TNF-α, tumour necrosis factor-α; IL-6, interleukin-6; hs-CRP, high sensitivity CRP; MCP-1, macrophage chemoattractant protein-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; E-selectin, endothelial selectin; P-selectin, platelet-selectin; vWF, von Willebrand factor; sTM, soluble thrombomodulin.

state with the elaboration of tissue factor which is acritical and potent factor in the initiation of coagulation in both physiologic and pathologic conditions (Grignani and Maiolo, 2000) (Figure 1). The HIV+/ACS+ group were also found to have greater thrombus burden on angiography in a previous study (Becker et al., 2010) which may, in part, be related to this well documented relationship between inflammation and thrombosis (Frostegard et al., 1999) (Figure 1). Furthermore, we showed that this group of patients also had higher rates of in-stent restenosis (Becker et al., 2010) which is thought to be related to a heightened inflammatory state during percutaneous coronary intervention (PCI) (Hsue et al., 2004). There was no difference in the inflammatory markers at six months but levels of the pro-atherogenic chemokine MCP-1 was significantly higher in HIV+ patients. MCP-1 is a chemokine responsible for the recruitment of monocytes to sites of inflammation where they promote atherosclerotic lesions and plaque vulnerability (de Lemos et al., 2003). Levels of MCP-1 have been shown to correlate with carotid intima-media thickness, a surrogate marker of atherosclerosis in HIV patients (Joven et al., 2006). Furthermore, MCP-1 is thought to play an important role in the pathogenesis of restenosis after PCI (Jianli and Kolattukudy, 2009). An unexpected finding was that ICAM-1 and sE-selectin levels were significantly higher at six months in the ACS+/ HIV- group. This may have been due to selection bias as a greater number of HIV+ patients died prior to the six month follow up compared to those without HIV; thus selecting out the sicker patients who may have had a greater degree of endothelial activation. With respect to the HIV+ group comparison could only be made at baseline as the HIV+ alone group did not have markers performed at 6 months. At baseline, IL-6 and hs-CRP were significantly higher in the ACS+/ HIV+ group but when comparing the baseline levels of inflammatory cytokines of the HIV+ alone group with the 6 month levels of the ACS+/ HIV+ group (that is both free of a thrombotic event), there was no difference. All markers of endothelial activation were significantly higher at baseline in the HIV+ alone group despite having a lower pro-inflammatory burden. This may be explained by the fact that endothelial cell activation in HIV has many triggers with pro-inflammatory cytokines being only one of them. Other known mechanisms include lipid disorders associated with HIV infection (Grinspoon and Carr, 2005), viral protein-related endothelial activation (Ren et al., 2002) and direct HIV infection of the endothelium and vascular smooth muscle cells (Conald et al., 1995). Of note, is that patients in the HIV+ alone group had lower CD4 cell counts with a higher percentage of patients with

AIDS; all factors which favour a greater degree of endothelial cell perturbation (Seigneur, 1997). A proposed link between HIV, inflammation and thrombosis is presented in Figure 1.

Although the study constitutes one of the largest prospective analyses on treatment-naïve patients with ACS, certain limitations need to be acknowledged. Firstly, given the nature of clinical presentations and our planned analyses, we were unable to completely match case-controls according to age, sex and risk parameters. The sample size in each group is relatively small resulting in a lack of power to detect small differences between the groups which may have been significant. In addition, not all patients had repeat testing at 6 months which would have influenced the validity of the 6 month markers of inflammation and endothelial cell activation in the patients who had thrombotic events. Wherever possible, however, we have adhered to the recently published STROBE guidelines in our reporting of study data (von Elm et al., 2007).

Conclusions

Treatment-naïve black South African patients with HIV and ACS have evidence of a heightened pro-inflammatory state and greater degree of endothelial cell activation compared to HIV negative patients, factors which may play a direct or indirect role in the path-genesis of thrombosis and ACS in this otherwise low risk population. The role of MCP-1 as a potential risk factor and marker of HIV-associated CAD and restenosis needs to be further elucidated.

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CHAPTER NINE: DISCUSSION AND CONCLUSIONS

9.1 DISCUSSION

The “Heart of Soweto” study (HOS) accumulated detailed clinical data over a one year period on the presentation, investigations and treatment of new and old patients with cardiovascular disease from the large urban area of Soweto (2006). Data from this study clearly supports the theory that this area, a microcosm of other urban areas in South Africa, is currently in a state of epidemiologic transition, with a high prevalence of modifiable cardiovascular risk factors for atherosclerosis and a combination of infectious and non-communicable forms of heart disease, often with late clinical presentations. The presence of a large component of non-communicable cardiovascular disease (e.g. hypertensive heart disease and CAD) and its common antecedent, type 2 diabetes mellitus, is consistent with a broadening spectrum of cardiovascular disease in this population.

In view of its near historical absence in black South Africans, the number of documented cases of CAD in blacks (77 or 5% of all new cases of heart disease in the HOS study) is consistent with the finding of a high burden of modifiable risk factors in the population. Further evidence for an emerging epidemic of cardiovascular disease, in particular CAD, is presented in Chapter 4.This epidemiologic study demonstrates a substantial increase in the number of patients diagnosed with ACS at Baragwanath Hospital in recent years. Fifty years ago, three such patients were identified per year; about 25 years ago, five

patients were diagnosed per year; in 2004 there were 64 diagnoses; and in 2006, 77 black South Africans were diagnosed ("HOS" study). Consistent with a population in epidemiologic transition, there was a more than ten-fold increase in the rate of coronary events over two decades, paralleled by increased rates of modifiable risk factors.

With the urban population of Soweto in epidemiologic transition and facing an HIV epidemic (Chapter 1 section 3), the future potential for an increase in cardiovascular morbidity and mortality is great. Furthermore, given that a greater proportion of HIV patients will be exposed to HAART in the future and that HAART has been associated with adverse metabolic complications and a greater risk of ACS (Chapter 1 section 5.3.3), the potential problem may be amplified. For these reasons a baseline understanding of the mechanisms relating to treatment-naïve HIV-related CAD is important if much needed guidelines regarding primary prevention and treatment are to be implemented. It is in this context that a focused study of this group of patients was undertaken.

When looking at the demographic and clinical profiles of treatment-naïve black South Africans with HIV compared to their HIV negative counterparts, several differences were observed. Classical risk factors for ACS were more prevalent in the HIV-negative group, which comprised an older population with more hypertension, diabetes, hyperlipidaemia, abdominal obesity and other "non-traditional" risk factors. Cigarette smoking, however, was more prevalent in the HIV group and consistent with studies from developed countries. HIV patients also had significantly lower HDL cholesterol levels, an independent risk factor for CAD, thrombosis and ACS.

How do we interpret these results relative to previous documented reports on ACS in HIV patients? Consistent with other studies from the developed world (see Chapter 1 section 5.2), HIV patients were younger and predominantly male, with a high proportion of cigarette smokers and low HDL levels (thought to be due to an impaired reverse cholesterol transport pathway mediated by the HIV regulatory protein nef) (see Chapter 1 section 5.3.2). In contrast to findings in the developed world, none of the treatment-naïve HIV patients were intravenous drug abusers and none had opportunistic infections or HIV-related malignancies - factors known to confer a higher thrombotic risk (see Chapter 1 section 5.5). The degree of immunosuppresion in HIV patients did not correlate with the risk of ACS. In terms of angiographic features, HIV patients had less atherosclerotic burden, with significantly more angiographically normal infarct related arteries (IRA), probably reflecting the younger age of the group with less classical risk factors.

What was apparent in the HIV patients was a higher degree of thrombus burden in the IRA (see Pictures 1 and 2), suggesting a prothrombotic state. Percutaneous coronary intervention was the preferred method of revascularisation of the IRA in both groups, with good procedural success. However, this was complicated by a high incidence of diffuse proliferative in-stent restenosis (see Methods Appendix, Figure 1.5) at follow up in HIV patients receiving bare metal stents (see Pictures 3 and 4). This finding is consistent with reports on in-stent restenosis (ISR) in HIV patients from the developed world (see Chapter 1 section 5.2). The pathogenesis of ISR in HIV patients is thought to be due to the heightened inflammatory state at the time of PCI (see Chapter 1 section

5.2), including increased circulating levels of the pro-inflammatory chemokine, MCP-1 (202).

Drug eluting stents (DES) are currently the preferred treatment for in-stent restenosis, but no data exists on their efficacy or safety in the setting of HIV infection. In this study, four HIV patients with diffuse proliferative in-stent restenosis received DES, with good procedural success and no need for target lesion revascularisation during follow up.

In terms of the overall clinical outcomes, MACE rates were higher in the HIV group, driven by increased rates of TLR but not death. Although the rates of death were not statistically significant, there was a higher death rate in the HIV group (30% vs. 17%) (Chapter 5) probably relating to direct complications of HIV infection as 67% of these deaths occurred after the index event and were not related to cardiovascular causes.

In view of the following clinical and angiographic features in the HIV patients, a prothrombotic state was suggested: minimal traditional risk factors; low atherosclerotic burden; high thrombotic burden. A prothrombotic state has been well described in HIV patients in the developed world and is thought to be multifactoral in nature (Table 1.9). With respect to the findings of the thrombotic screens in this study, the ACS+: HIV+ group (Group 1) had greater evidence of thrombophilia compared to the ACS+: HIV- group (Group 2). Protein C (PC) levels were significantly lower and factor VIII levels significantly higher in the ACS+: HIV+ group. PC deficiency is a well-known risk factor for venous thromboembolism (VTE) (see Chapter 1 section 5.5) and confers an increased

risk of around seven-fold; but this has also been described in the pathogenesis of myocardial infarction in young patients with otherwise normal coronary arteries, such as this HIV group. PC deficiency results in increased factor IXa and Xa activity and a hypercoagulable state (see Methods Appendix, Figure 1.6).The mechanism of PC deficiency in the setting of HIV infection is multifactoral in nature, including: decreased synthesis, increased metabolism, as well as low grade disseminated intravascular coagulation (DIC) with consumptive coagulopathy (see Chapter 1 section 5.5).

Increased factor VIII is known to elevate the risk of VTE, but little is known about its relationship to arterial thrombosis or HIV infection; its pathogenic role is thus questionable. There was no evidence of hyper-reactive platelets in any of the groups studied and the incidence of aspirin resistance was similar between the two groups.

With respect to antiphospholipid antibodies (aPL), HIV infection is known to be associated with increased frequencies of aPL and the antiphospholipid syndrome (APS) is found more commonly in populations with ACS, which have a low burden of conventional risk factors with little or no evidence of atherosclerotic disease (see Chapter 1 section 5.5). Significant differences were found in terms of aPL frequencies between the two groups, with HIV patients having higher frequencies of anticardiolipin (aCL) IgG and antiprothrombin (aPT) IgG antibodies. The incidence of APS was, however, similar in the groups and the the presence of APS did not predict any of the clinical or angiographic outcomes.

Comparison between the two HIV groups' thrombotic profiles (ACS+: HIV+ (Group 1) and ACS-: HIV+ (Group 3)) was performed to control for HIV status. The patients were well matched with respect to age and sex, but the ACS+: HIV+ group were less immunocompromised, with higher CD4 counts and no evidence of opportunistic infections or AIDS related malignancies; this implied that immune dysregulation did not seem to play a significant role in the development of arterial thrombosis, unlike its reported role in VTE. The ACS+: HIV+ group had significantly lower PC levels compared to the ACS-: HIV+ group, despite being less immunocompromised; this made the finding even more relevant, as PC levels in HIV tend to decrease with worsening immunosuppresion (see Chapter 1 section 5.5).

With respect to aPL frequencies, similar to a report by Loizou et al. (see Chapter 1 section 5.5), who investigated HIV positive black South Africans, a different pattern of aPL expression was found in the HIV patients compared to recent studies investigating a predominantly Caucasian population (which reported aCL to be positive in 36-88%, anti-ß$_2$-GP-1 in 4-27%, and aPT in 2-12% of patients) (see Chapter 1 section 5.5). In keeping with the study by Loizou et al., a high prevalence of aPT IgG antibodies was found in the HIV group, with 87% in the ACS+ : HIV+ group and 97% in the ACS- : HIV+ group. The rates of aCL and anti-ß$_2$-GP-1 antibodies seen in our black HIV population was similar to those described in the recent studies on Caucasian patients described above. There were no significant differences when comparing the aPL frequencies within the HIV group, but analysis of the actual titres revealed higher levels of anti-ß$_2$-GP-1 IgM as well as aPT (IgG,IgM,IgA) in the ACS- : HIV+ group; this was possibly due to a greater

degree of immunosuppresion and immune dysregulation, which suggests that the higher frequencies of aPL seen in HIV patients are an epiphenomenon of HIV infection, rather than causally linked to thrombotic events. Despite case reports of APS in HIV patients (see Chapter 1 section 5.5), the aPL often present in infectious diseases are not usually associated with thrombotic complications. These aPL could be induced by disturbances in regulation of cellular and humoral immunity, as a secondary consequence of HIV infection.

Another mechanism considered important in the pathogenesis of thrombosis is the link between inflammatory cytokines and their activation of endothelial cells and monocytes (see Chapter 8, Figure 1.8). Inflammatory markers were found to be higher in the HIV+ : ACS+ group compared to the ACS+ : HIV- group, at baseline, with significantly higher levels of the pro-inflammatory cytokines TNF-α and IL-6. There was no evidence of acute infection at the time the assays were performed, making it unlikely that the difference in markers could be explained by an underlying, confounding infectious process, which has been shown to be a risk factor in the precipitant of ACS. This pro-inflammatory state is likely related to the effects of the HIV virus on CD4 cell and macrophage activation, with consequent elaboration of pro-inflammatory cytokines; this may, in turn, lead to accelerated atherosclerosis and a prothrombotic state with the elaboration of tissue factor (Figure 1.8) and activation of the extrinsic coagulation cascade. This heightened inflammatory state, together with the factors mentioned earlier, may (to some extent) explain the finding of large thrombus burden in the IRA's of HIV positive patients and the higher degree of in-stent restenosis.

There was no difference in the inflammatory markers at six months, but levels of the pro-atherogenic chemokine MCP-1 were significantly higher in the HIV group. MCP-1 is a chemokine responsible for the recruitment of monocytes to sites of inflammation, where they promote atherosclerotic lesions and plaque vulnerability (see Chapter 1 section 5.3). MCP-1 levels have been shown to correlate with surrogate markers of atherosclerosis in HIV patients (see Chapter 8) and the molecule is considered a candidate marker for atherosclerosis. In addition, elevated MCP-1 levels in the HIV group in this study probably contributed to the high rates of in-stent restenosis seen in patients who received bare metal stents.

Baseline endothelial markers between the ACS+ : HIV+ and ACS- : HIV+ group also showed a significant difference, with higher levels of VCAM-1 in the HIV group. Increased levels of this molecule have been shown to occur with stimulation of endothelial cells by pro-inflammatory cytokines (see Figure 1.8) and it has a strong predictive value of future cardiovascular death. With respect to the HIV group (ACS+ : HIV+ and ACS- : HIV+), comparison could only be made at baseline, as the ACS- : HIV+ group did not have markers performed at 6 months.

Levels of IL-6 and hs-CRP were significantly higher in the ACS+ : HIV+ group, but when comparing the baseline levels of the ACS- : HIV+ group with the 6 month levels of the ACS- : HIV+ group (i.e. both free of a thrombotic event), there was no difference. All markers of endothelial activation were significantly higher in the ACS- : HIV+ group,

despite the group having a lower inflammatory burden. This may be explained by the fact that endothelial cell activation in HIV infection has many triggers, including HIV-associated lipid disorders, viral protein related endothelial activation and direct infection of the endothelium with the virus (see Chapter 1 section 5.3.2, Figure 1.8). In addition, the ACS- : HIV+ group had a greater degree of immunosupression, with lower CD4 counts and a greater number of patients with AIDS defining criteria, all of which favour a greater degree of endothelial cell activation.

9.2 LIMITATIONS OF THE STUDY

This study has a number of limitations that require comment. Although this represents a historically large cohort of treatment-naïve HIV positive patients presenting with an ACS, the overall study cohort was small and thus underpowered to detect small differences between the groups. Age specific MI rates in black patients from Soweto are not known and we were thus unable to determine whether the incidence of ACS is higher in treatment-naïve HIV patients compared to age-matched HIV negative patients.

Incomplete data, particularly at the 6-month mark (due to a relatively high death rate, particularly in the HIV group), resulted in further weakening of statistical power. For example, not all patients receiving BMS underwent repeat angiography, which affected the reliability of the data regarding in-stent restenosis. In addition, not all patients underwent initial thrombotic screening and a significant number were not eligible for antiphospholipid assays and inflammatory markers at 6 months, which weakened the

statistical power and significance of these tests. Furthermore, imperfect matching of the two HIV groups, with respect to degree of immunosuppresion, made direct comparison of the thrombotic and inflammatory profiles difficult, but wherever possible we have adhered to the recently published STROBE guidelines (203) in our reporting of study data.

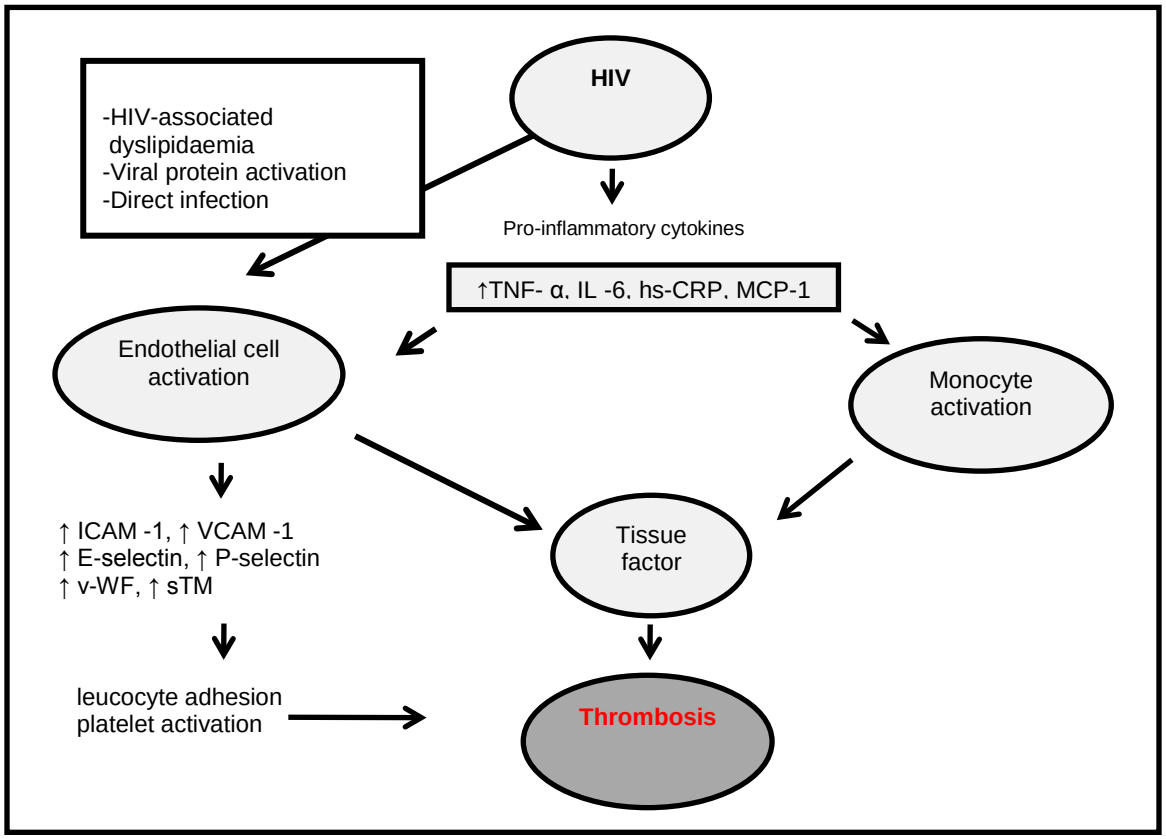
9.3 CONCLUSIONS

Soweto, a large predominantly black urban area in South Africa, is in a state of epidemiologic transition with an increasing prevalence of modifiable cardiovascular risk factors and ischaemic heart disease. Treatment-naïve HIV positive black patients presenting with ACS in Soweto have different clinical and angiographic features compared to the HIV negative population. The patients were younger, more commonly male, with high rates of smoking, low HDL levels and less atherosclerotic burden, but a higher thrombotic burden, suggesting a prothrombotic state. This was confirmed by lower Protein C levels, higher factor VIII levels and a greater degree of inflammatory burden and endothelial cell activation. The exact pathogenic role of HIV, independent of associated modifiable and non-modifiable risk factors, is difficult to determine, but may be important as a contributory factor in an already “vulnerable” patient. A proposed link between HIV and atherothrombosis is depicted in Figure 9.1. Importantly, we identified modifiable risk factors in the HIV group. Smoking may play a crucial role in the pathogenesis of ACS in these otherwise seemingly low risk patients and remains an important target for cardiovascular risk reduction.

The role of HDL in the pathogenesis and prevention of HIV-associated CAD needs to be further defined, as does the role of drug eluting coronary stents in the prevention of in-stent restenosis. Thrombophilic screening in HIV positive patients with minimal coronary risk factors, presenting with ACS, may be justified to aid in decision making regarding anti-coagulation as part of secondary prevention. Cardiovascular risk assessment and

appropriate primary prevention should be an important component in the management of HIV patients, regardless of treatment status.

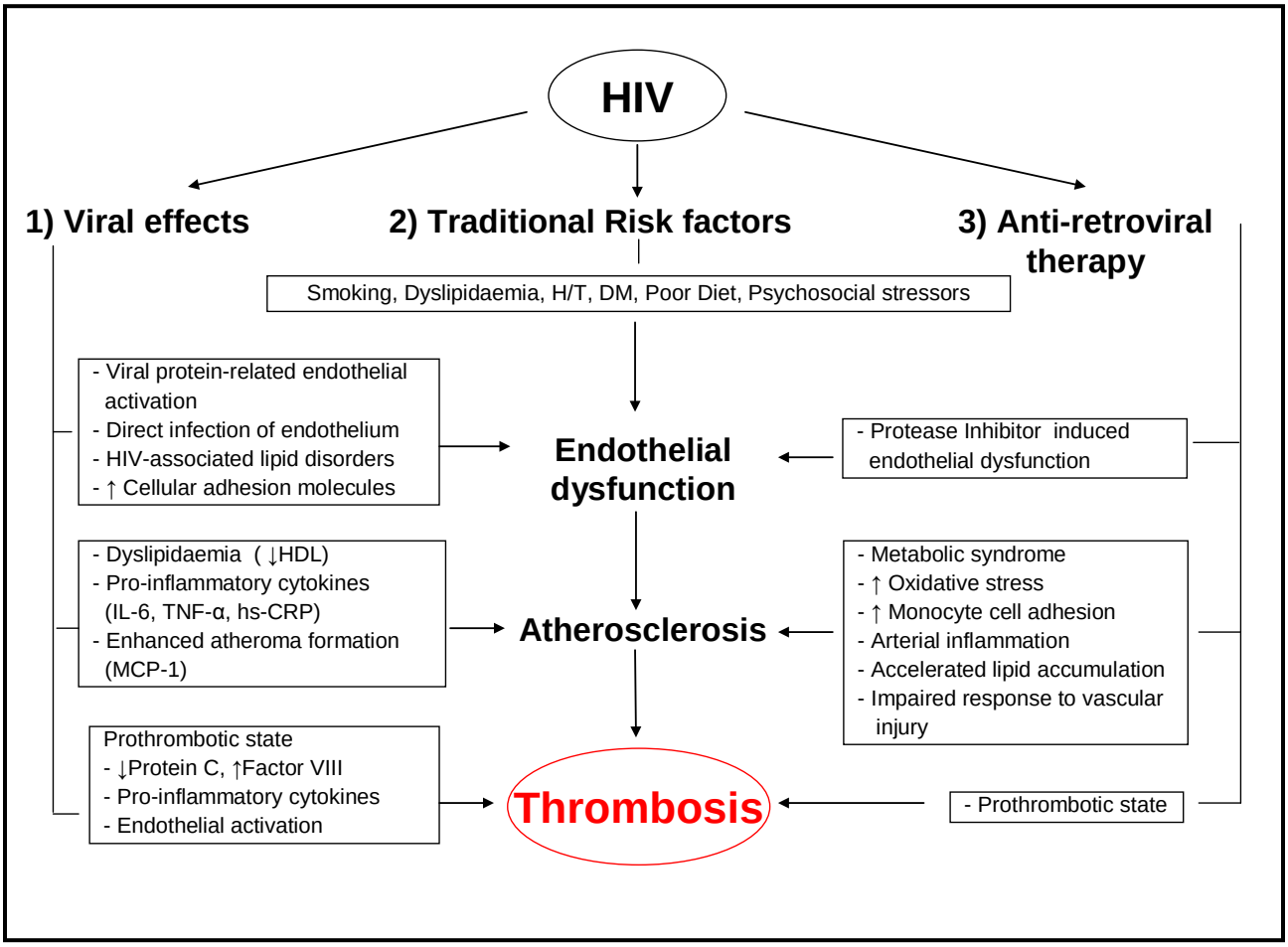
Figure 9.1: A proposed link between HIV, inflammation and thrombosis



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Key: TNF-α - tumour necrosis factor-α; IL-6 -, interleukin-6; hs-CRP -, high sensitivity CRP; MCP-1 -, macrophage chemoattractant protein-1; ICAM-1 -, intercellular adhesion molecule-1; VCAM-1 -, vascular cell adhesion molecule-1; E-selectin -, endothelial selectin; P-selectin -, platelet-selectin; vWF -, von Willebrand factor; sTM -, soluble thrombomodulin

Figure 9.2: HIV and atherothrombosis



Key: DM - diabetes mellitus; HDL - high density lipoprotein; hs-CRP - high sensitivity C reactive protein; H/T - hypertension; IL-6 - interleukin-6; MCP-1 - macrophage chemoattractant protein-1; TNF-α - tumour necrosis factor-α

9.4 THE WAY FORWARD

The studies in this thesis have shown that the cardiovascular risk facing treatment-naïve black South Africans with HIV is multifactoral, involving traditional risk factors and HIV-related risk factors (Figure 1.9). Furthermore, anti-retroviral drugs (ARV's) are now relatively freely available at all major state hospitals in South Africa, which will result in an increased exposure of these patients to drugs which, while vitally important to the well-being of HIV patients, are potentially deleterious from a cardiovascular perspective and add a third arm to the potential risk facing these patients (Figure 9.1). According to the National Strategic Plan (Table 1.1), a significant increase in the utilisation of ARV's is planned, with an ambitious target of 420 000 new adults initiated on ARV's by 2011.

Having the baseline data on modifiable and non-modifiable risk factors facing treatment-naïve patients, several important areas for future research arising from this study have emerged and in order to manage the potential future epidemic of CAD in HIV positive patients decisively, three important data sets are required:

1) appropriate cardiovascular risk screening models;
2) effective primary prevention strategies;
3) appropriate treatment of ACS and optimisation of PCI.

1) Screening of cardiovascular risk in HIV patients

Prediction equations for CAD risk are useful tools that inform clinicians and patients about the absolute risk for developing CAD. A basic principle in CAD prevention is that

the intensity of risk-reducing interventions should be based on the individual patient's absolute CAD risk. Given the increased life expectancy as a result of HAART, in the future more patients will experience complications not related to HIV per se and will reach an age at which they are at increased risk for developing CAD. There are currently no CAD risk calculations specifically designed for HIV positive patients; in fact, the most commonly used risk calculator, The Framingham risk score (see Appendix), was based on a North American Caucasian population. This has questionable validity in black South Africans as a whole, regardless of HIV status. Although traditional risk factors may operate in the same manner in HIV patients as in the general population, there is still a need to identify and evaluate HIV-specific CAD risk factors and develop new HIV-specific CAD prediction equations for adults, adolescents and children in South Africa.

Ideally, what is required is a large multi-center prospective study using a proportional-hazards regression model that captures a core set of traditional and HIV-specific variables; these should all be collected at standard intervals in HAART-naïve and treated HIV-infected children, adolescents and adults to determine (with certainty) the optimal HIV-specific risk prediction algorithm and the relative contribution of HIV infection and HAART to CAD in this population. Ideally, such a study would obtain fundamental data on:

1) the relative contribution of HIV infection (replication, altered immunity and inflammation), HAART component(s), central adiposity, peripheral lipoatrophy and metabolic dysregulation to CAD risk in HIV

2) the safety and efficacy of standard treatments (diet, weight loss, physical activity) and more intensive therapies (lipid, glucose and blood pressure lowering therapies, as well as optimal ARV regimens) for CAD in HIV.

The starting point for the development of a CAD risk prediction equation in HIV patients would be a comprehensive medical history and clinical examination, with standardised collection of key predictor (independent) risk factors: age, sex, fasting lipids, systolic blood pressure, history of diabetes mellitus, fasting or post prandial glucose levels, and use of tobacco. Most existing equations predict CAD morbidity and mortality. Such end points need to be defined a priori and captured in data collection (dependent variables). In terms of HIV, the challenge is how to allow for HIV-specific factors, i.e. immunological state, levels of inflammation, duration of disease and types of ARV's used.

2) Primary prevention strategies

A central tenet of preventing CAD is that the intensity of risk-reducing interventions should be based on the level of CAD risk. The first key step in offering primary prevention to HIV patients, therefore, is to: estimate the underlying CAD risk (discussed in the section on screening above); then to evaluate various therapies, which have been shown to be effective in the HIV-negative population, such as aspirin, statins, anti-hypertensives and various less atherogenic ARV regimens. Important modifiable risk factors found in this study were cigarette smoking and low HDL levels.

2a) Smoking

Smoking is highly prevalent among patients with HIV and more prevalent than in the general population (see Chapter 1 section 5.3.2), as is the case with Black South Africans. Aside from having a history of CAD, current cigarette smoking is the most powerful predictor of CAD events among patients with HIV; therefore, preventing individuals with HIV from starting to smoke is critical, because stopping smoking remains a formidable challenge. Physicians of patients with HIV are less likely than non-HIV healthcare providers to ascertain smoking status and encourage quitting (204).

Specific research aimed at encouraging smoking abstinence and encouraging quitting is required and appropriate research questions would be:

- What is the general attitude of healthcare workers and patients regarding smoking and in particular to smoking cessation?
- What are the factors that perpetuate the smoking habit in HIV patients, e.g. lack of healthcare provider awareness and/or encouragement to stop smoking, lack of social network support, lack of access to proven smoking cessation strategies or fatalism.
- Do simple, cost effective measures such as the "5A" approach make a difference in the rates of smoking cessation? [5A approach (204): Ask about tobacco use; Advise to quit; Assess willingness to make a quit attempt; Assist with quit attempt; Arrange for follow up].

- Specific research regarding the safety and efficacy of smoking cessation pharmacotherapy in HIV patients and, in particular, pharmacokinetic and pharmacodynamic studies to evaluate the effects of smoking cessation on ART and drug interactions between ART and smoking cessation pharmacotherapy.

2b) HDL

A strong inverse relationship between HDL cholesterol levels and risk for CAD is well established in the non-HIV population, but no data currently exists on its importance in HIV. There are no current HIV-specific guidelines in South Africa; current management is based on evidence from the non-HIV population and utilises guidelines from either South African Lipid Guidelines or those from the Third National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (NCEP ATP III) (205).

Data from this thesis show significantly lower HDL levels in the HIV patients having an ACS and this may be an important target for primary prevention. Managing dyslipidaemia in HIV patients is potentially problematic, due to drug interactions, relative lack of efficacy and the problem of increasing pill burden in patients receiving multi-drug regimens. Ideally, as part of a larger prospective study on CAD risk (see section 1 above), the efficacy of various lipid lowering agents on raising HDL levels (and lowering LDL and triglycerides), in terms of cardiovascular outcomes, should be performed. However, costs and logistics may be prohibitive.

Prospective studies on progression of a surrogate marker, such as carotid intima media thickness would be useful. This would aid in formulating HIV-specific lipid guidelines appropriate to the South African context.

3) Optimising percutaneous coronary intervention

Percutaneous coronary intervention (PCI) is the current standard of treatment for patients with ACS. Patients studied in this thesis, both HIV positive and negative had equally good acute results with low complication rates. What was of concern, however, was the high incidence of in-stent restenosis with bare metal stents (BMS) in the HIV group, which resulted in an unacceptably high incidence of target lesion revascularization. Drug eluting stents (DES) are the current standard of treatment for in-stent restenosis and were used effectively in four HIV patients with good success and no complications. Despite this, no formal data exists on their safety and long term efficacy in the setting of HIV.

A prospective, randomised controlled trial assessing the long term success of DES vs. BMS in HIV patients is indicated, but due to cost constraints, an interim solution would be to study this as part of a National Cath Lab registry, from which appropriate data could be drawn. In addition, valuable data regarding the efficacy and safety of Clopidogrel, an anti-platelet agent used post-PCI, could be obtained. Currently, Clopidogrel is used for a period of at least 1 month post BMS implantation and 6 months post DES implantation. The appropriate duration of Clopidogrel use post-PCI in HIV

patients is unknown, but theoretically it may be required longer term due to the association with the prothrombotic state. In addition, Clopidogrel utilises the cytochrome P450 system and may interact with various important medications commonly prescribed in HIV patients, especially protease inhibitors, which raises questions about the safety of the drug.

With the anticipated increase in CVD facing South Africa, owing to the many reasons detailed in this thesis, further research projects that are appropriate to the South African context will be vital in order to further quantify the need and explore cost-effective ways to provide primary and secondary prevention in order to effectively deal with the burden of epidemiological transition as well as the cardiovascular burden likely to be imposed by the HIV pandemic.

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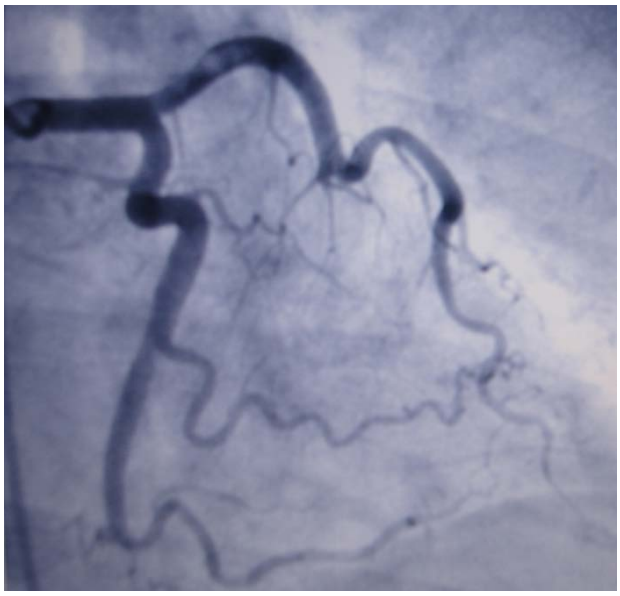
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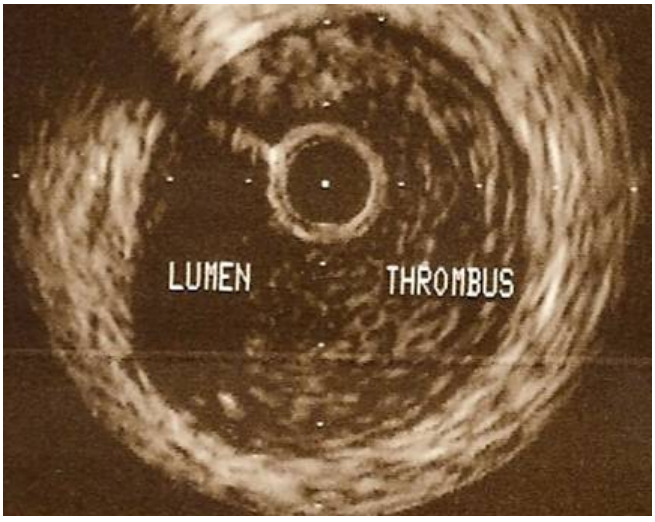
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PICTURES

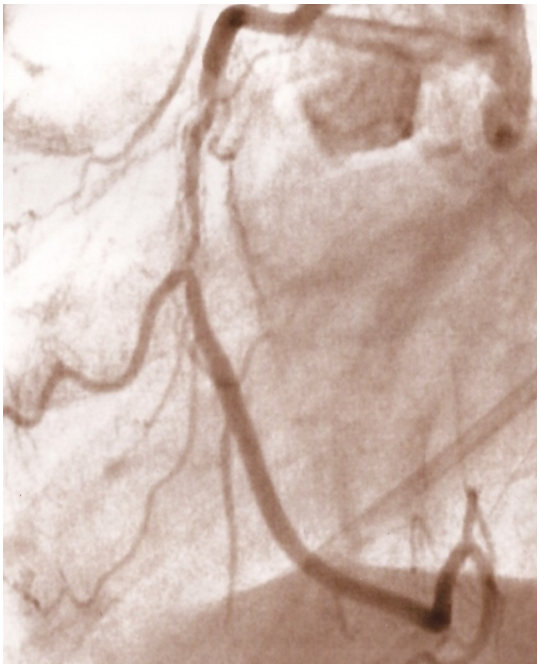
Picture 1. Angiographic image of thrombus in an otherwise angiographically normal left anterior descending coronary artery in a 43-yearold HIV positive male presenting with an ST-elevation MI.



Picture 2. Intravascular ultrasound (IVUS) image of thrombus in an otherwise angiographically normal left anterior descending coronary artery depicted in Picture 1.



Picture 3. Diffuse proliferative In-stent restenosis (Type 3) in the right coronary artery of a 32-year-old HIV positive male 15 months post bare metal stent implantation.



Picture 4. Intravascular ultrasound (IVUS) image of diffuse proliferative In-stent restenosis (Type 3) in the patient depicted in Picture 3.



APPENDIX

Human Research Ethics Committee clearance documents

Protocol approval document

Statement of originality documents

Framingham risk score

Anti-retroviral drugs currently available in South Africa and common adverse events

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Becker

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M040702**
PROJECT	Acute Coronary Syndromes in Black SA Patients with Human Immunodeficiency Virus Infection Clinical Features and...
INVESTIGATORS	Dr A Becker
DEPARTMENT	School of Clinical Medicine
DATE CONSIDERED	04.07.30
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 04.10.10 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof K Sliwa

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
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DR AC BECKER
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2196

APPLICATION NUMBER 9100815K
STATUS (DEG 29) (MD012) PZZ

2005-10-28

Dear Dr Becker

Approval of protocol entitled Acute Coronary Syndromes in Black South African Patients with Human Immunodeficiency Virus Infection

I should like to advise you that the protocol and title that you have submitted for the degree of Doctor Of Philosophy (Part-Time) have been approved by the Postgraduate Committee at its recent meeting. Please remember that any amendment to this title has to be endorsed by your Head of Department and formally approved by the Postgraduate Committee.

Dr. K Sliwa-Hahnle has/have been appointed as your supervisor/s. Please maintain regular contact with your supervisor who must be kept advised of your progress.

Please note that approval by the Postgraduate Committee is always given subject to permission from the relevant Ethics Committee, and a copy of your clearance certificate should be lodged with the Faculty Office as soon as possible, if this has not already been done.

Yours sincerely

S Benn (Mrs)
Faculty Registrar
Faculty of Health Sciences

Telephone 717-2075/2076

Copies - Head of Department_____Supervisor/s

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The University seeks to serve South Africa by furthering access to equal opportunity while striving for excellence in teaching, learning and research

CHAPTER 3: SPECTRUM OF HEART DISEASE AND RISK FACTORS IN A BLACK URBAN POPULATION IN SOUTH AFRICA: THE "HEART OF SOWETO STUDY"

Statement of Originality

NAME	RESPONSIBILITY
Karen Sliwa, University of the Witwatersrand	Conceived and designed the research Acquired the data Analysed and interpreted the data Handled funding and supervision Drafted the manuscript
David Wilkinson, University of Queensland	Conceived and designed the research Analysed and interpreted the data Drafted the manuscript
Craig Hansen, University of Queensland	Conceived and designed the research Analysed and interpreted the data Performed statistical analysis
Lucas Ntyintyane, University of the Witwatersrand	Conceived and designed the research Acquired the data Analysed and interpreted the data Drafted the manuscript
Kemi Tibazarwa, University of the Witwatersrand	Conceived and designed the research Acquired the data Analysed and interpreted the data Drafted the manuscript
Anthony Becker, University of the Witwatersrand	Acquired the data Analysed and interpreted the data Drafted the manuscript
Simon Stewart, Baker Research Institute	Conceived and designed the research Analysed and interpreted the data Performed statistical analysis Handled funding and supervision Drafted the manuscript
Candidate: I declare that this work is wholly my own accept where acknowledged as being the work of others (as listed above). I also acknowledge the contribution of others (as listed above) to this work in this Statement of Originality. Dr Anthony Becker 2011	Principal advisor: I hereby certify that all co-authors have provided their consent for the inclusion of the paper in the thesis and that the co-authors accept the candidate's contribution to the paper as described in this Statement of Originality. Professor Karen Sliwa 2011

CHAPTER 4: EMERGING EPIDEMIC OF CARDIOVASCULAR DISEASE AMONG URBAN AFRICANS: ACUTE CORONARY SYNDROME AT BARAGWANATH HOSPITAL, SOWETO

Statement of Originality

NAME	RESPONSIBILITY
Christine Amira, University of the Witwatersrand	Conceived and designed the research Acquired the data Analysed and interpreted the data Drafted the manuscript
Lucas Ntyintyane, University of the Witwatersrand	Conceived and designed the research Acquired the data Analysed and interpreted the data Drafted the manuscript
David Wilkinson, University of Queensland	Conceived and designed the research Analysed and interpreted the data Drafted the manuscript
Simon Stewart, University of the Witwatersrand	Conceived and designed the research Analysed and interpreted the data Drafted the manuscript
Anthony Becker, University of the Witwatersrand	Conceived and designed the research Acquired the data Analysed and interpreted the data Drafted the manuscript
Elena Libhaber, University of the Witwatersrand	Analysed and interpreted the data Performed statistical analysis Drafted the manuscript
Karen Sliwa, University of the Witwatersrand	Conceived and designed the research Acquired the data Analysed and interpreted the data Handled funding and supervision Drafted the manuscript
Candidate: I declare that this work is wholly my own accept where acknowledged as being the work of others (as listed above). I also acknowledge the contribution of others (as listed above) to this work in this Statement of Originality. Dr Anthony Becker 2011	Principal advisor: I hereby certify that all co-authors have provided their consent for the inclusion of the paper in the thesis and that the co-authors accept the candidate's contribution to the paper as described in this Statement of Originality. Professor Karen Sliwa 2011

CHAPTER 5: ACUTE CORONARY SYNDROMES IN TREATMENT NAÏVE BLACK SOUTH AFRICANS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Statement of Originality

NAME	RESPONSIBILITY
Anthony Becker, University of the Witwatersrand	Conceived and designed the research Acquired the data Analysed and interpreted the data Performed statistical analysis Drafted the manuscript
Karen Sliwa, University of the Witwatersrand	Conceived and designed the research Analysed and interpreted the data Handled funding and supervision Drafted the manuscript
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CHAPTER 6: THE THROMBOTIC PROFILE OF TREATMENT NAÏVE BLACK SOUTH AFRICANS WITH HIV AND ACUTE CORONARY SYNDROMES

Statement of Originality

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Anthony Becker , University of the Witwatersrand	Conceived and designed the research Acquired the data Analysed and interpreted the data Performed statistical analysis Drafted the manuscript
Barry Jacobson , University of the Witwatersrand	Conceived and designed the research Analysed and interpreted the data Handled funding and supervision Drafted the manuscript
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Karen Sliwa , University of the Witwatersrand	Conceived and designed the research Analysed and interpreted the data Handled funding and supervision Drafted the manuscript
Simon Stewart , University of the Witwatersrand	Conceived and designed the research Analysed and interpreted the data Drafted the manuscript
Elena Libhaber, University of the Witwatersrand	Analysed and interpreted the data Performed statistical analysis Drafted the manuscript
Rafique Essop, University of the Witwatersrand	Conceived and designed the research Analysed and interpreted the data Handled supervision Drafted the manuscript
Candidate: I declare that this work is wholly my own accept where acknowledged as being the work of others (as listed above). I also acknowledge the contribution of others (as listed above) to this work in this Statement of Originality. Dr Anthony Becker 2011	Principal advisor: I hereby certify that all co-authors have provided their consent for the inclusion of the paper in the thesis and that the co-authors accept the candidate's contribution to the paper as described in this Statement of Originality. Professor Karen Sliwa 2011

CHAPTER 7: ANTIPHOSPHOLIPID ANTIBODIES IN BLACK SOUTH AFRICANS WITH HIV AND ACUTE CORONARY SYNDROMES: PREVALENCE AND CLINICAL CORRELATES

Statement of Originality

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Karen Sliwa, University of the Witwatersrand	Conceived and designed the research Analysed and interpreted the data Handled funding and supervision Drafted the manuscript
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Simon Stewart, University of the Witwatersrand	Conceived and designed the research Analysed and interpreted the data Drafted the manuscript
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Candidate: I declare that this work is wholly my own accept where acknowledged as being the work of others (as listed above). I also acknowledge the contribution of others (as listed above) to this work in this Statement of Originality. Dr Anthony Becker 2011	Principal advisor: I hereby certify that all co-authors have provided their consent for the inclusion of the paper in the thesis and that the co-authors accept the candidate's contribution to the paper as described in this Statement of Originality. Professor Karen Sliwa 2011

CHAPTER 8: MARKERS OF INFLAMMATION AND ENDOTHELIAL ACTIVATION IN BLACK SOUTH AFRICANS WITH HIV AND ACUTE CORONARY SYNDROMES

Statement of Originality

NAME	RESPONSIBILITY
Anthony Becker, University of the Witwatersrand	Conceived and designed the research Acquired the data Analysed and interpreted the data Performed statistical analysis Drafted the manuscript
Elena Libhaber, University of the Witwatersrand	Analysed and interpreted the data Performed statistical analysis Drafted the manuscript
Karen Sliwa, University of the Witwatersrand	Conceived and designed the research Analysed and interpreted the data Handled funding and supervision Drafted the manuscript
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Candidate: I declare that this work is wholly my own accept where acknowledged as being the work of others (as listed above). I also acknowledge the contribution of others (as listed above) to this work in this Statement of Originality. Dr Anthony Becker 2011	Principal advisor: I hereby certify that all co-authors have provided their consent for the inclusion of the paper in the thesis and that the co-authors accept the candidate's contribution to the paper as described in this Statement of Originality. Professor Karen Sliwa 2011

FRAMINGHAM RISK SCORE to predict 10 year ABSOLUTE RISK of CHD EVENT

ST ALBANS & HEMEL HEMPSTEAD NHS TRUST : CARDIOLOGY DEPARTMENT

This risk assessment only applies to assessment for **PRIMARY PREVENTION of CHD**, in people who do not have evidence of established vascular disease.
Patients who *already* have evidence of vascular disease usually have a >20% risk of further events of over 10 years, and require vigorous **SECONDARY PREVENTION**.
People with a Family History of premature vascular disease are at higher risk than predicted; Southern Europeans and some Asians may have a lower risk in relation to standard risk factors.

STEP 1: Add scores by sex for Age, Total Cholesterol, HDL-Cholesterol, BP, Diabetes and Smoking. (If HDL unknown, assume 1.1 in Males, 1.4 in Females)

Age	M	F
30-34	-1	-9
35-39	0	-4
40-44	1	0
45-49	2	3
50-54	3	6
55-59	4	7
60-64	5	8
65-69	6	8
70-74	7	8

Total Cholesterol	M	F
< 4.1	-3	-2
4.1 – 5.1	0	0
5.2 – 6.2	1	1
6.3 – 7.1	2	1
7.2	3	3

HDL Cholesterol	M	F
< 0.9	2	5
0.9 - 1.16	1	2
1.17 - 1.29	0	1
1.30 - 1.55	0	0
≥1.56	-2	-3

Systolic BP	Diastolic BP				
Male	<80	80-84	85-89	90-99	≥100
<120	0	0	1	2	3
120-129	0	0	1	2	3
130-139	1	1	1	2	3
140-159	2	2	2	2	3
≥160	3	3	3	3	3
Female	**<80**	**80-84**	**85-89**	**90-99**	**≥100**
<120	-3	0	0	2	3
120-129	0	0	0	2	3
130-139	0	0	0	2	3
140-159	2	2	2	2	3
≥160	3	3	3	3	3

If Systolic and Diastolic BP fall into different categories, use score from higher category

Diabetes	M	F
No	0	0
Yes	2	4

Smoking	M	F
No	0	0
Yes	2	2

Categorisation of 10 year Risk of CHD Event	
Very Low risk	< 10%
Low risk	< 15%
Moderate risk	15-20%
High risk	> 20%

STEP 2: Use total score to determine Predicted 10 year Absolute Risk of CHD Event (Coronary Death, Myocardial Infarction, Angina) by sex

Total Score	≤-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	3	14	15	16	≥17
10 year Risk: Male		<2%	3%	3%	4%	5%	7%	8%	10%	13%	16%	20%	25%	31%	37%	45%	53%	63%	53%	53%
10 year Risk: Female	<1%	2%	2%	2%	3%	3%	4%	4%	5%	6%	7%	8%	10%	11%	13%	15%	18%	20%	24%	27%

STEP 3: Compare Predicted 10 year Absolute Risk with "Average" and "Ideal" 10 year Risks, to give Relative Risks

Age	30 - 34	35 - 39	40 - 44	45 - 49	50 - 54	55 - 59	60 - 64	65 - 69	70 - 74
"Average" Male	3%	5%	7%	11%	14%	16%	21%	25%	30%
"Ideal" Male	2%	3%	4%	4%	6%	7%	9%	11%	14%
"Average" Female	< 1%	< 1%	2%	5%	8%	12%	12%	13%	14%
"Ideal" Female	< 1%	1%	2%	3%	5%	7%	8%	8%	8%

"Ideal" risk represents
Total Cholesterol = 4.1 - 5.1
HDL = 1.2 (Male), 1.4 (Female)
BP < 120/80
No Diabetes, Non Smoker

People with an absolute risk of ≥20% should be considered for treatment: with a Statin to achieve a Total Cholesterol <5 and/or LDL cholesterol <3.2
with anti-hypertensives to achieve a BP ≤160/90 (ideally ≤140/80)

from Wilson PWF, et al Prediction of coronary heart disease using risk factor categories. Circulation 1998;97:1837-47 Dr John Bayliss

TABLE II. ARV AGENTS CURRENTLY AVAILABLE IN SOUTHERN AFRICA AND COMMON ADVERSE EVENTS

Generic name	Class of drug	Recommended dosage	Common adverse drug reactions*
Zidovudine (AZT)	NRTI	300 mg 12-hrly	**Bone marrow suppression,** gastro-intestinal (GI) upset, headache, myopathy, **hyperlactataemia**/steatohepatitis (medium potential)
Didanosine (ddI)	NRTI	400 mg/d (250 mg/d if <60 kg) taken on an empty stomach (enteric-coated formulation preferred)	Peripheral neuropathy, **pancreatitis,** nausea, diarrhoea, **hyperlactataemia**/steatohepatitis (high potential)
Lamivudine (3TC)	NRTI	150 mg 12-hrly or 300 mg/d	Anaemia, GI upset, myalgia, **hyperlactataemia**/steatohepatitis (low potential)
Stavudine (d4T)	NRTI	30 mg 12-hrly (note - higher doses for >60 kg no longer recommended owing to toxicity)	Peripheral neuropathy, lipo-atrophy, **hyperlactataemia**/steatohepatitis (high potential), **pancreatitis**
Abacavir	NRTI	300 mg 12-hrly or 600 mg/d	GI upset, **hypersensitivity reaction** 3%, **hyperlactataemia**/steatohepatitis (low potential)
Tenofovir (TDF)	NRTI	300 mg/d	Asthenia, headache, GI upset, **renal failure,** ddI concentrations increased 30 - 60%, reduced bone mineral density, **hyperlactataemia**/steatohepatitis (low potential)
Emtricitabine (FTC)	NRTI	200 mg/d	Headache, nausea, **hyperpigmentation,** hyperlactataemia/steatohepatitis (low potential)
Nevirapine (NVP)	NNRTI	200 mg/d for 14 days then 200 mg 12-hrly	**Rash, hepatitis**
Efavirenz (EFV)	NNRTI	600 mg at night	Rash, central nervous system symptoms, elevated transaminases
Nelfinavir†	PI	750 mg 8-hrly or 1 250 mg 12-hrly (take with food)	Diarrhoea, hyperglycaemia, dyslipidaemia
Indinavir	PI	800 mg 12-hrly with 100 mg ritonavir 12-hrly, no food restrictions	Kidney stones, unconjugated hyperbilirubinaemia, GI disturbances, hair loss, hyperglycaemia, headache, dyslipidaemia
Ritonavir	PI	600 mg 12-hrly - very rarely used as sole PI in adults	GI upset, circumoral and extremities paraesthesiae, diarrhoea, fatigue, hepatitis, taste perversion, hyperglycaemia, dyslipidaemia
Saquinavir (hard gel formulation)	PI	1 000 mg with ritonavir 100 mg 12-hrly (take with a fatty meal, or up to 2 hours after meal) or 1 600 mg with ritonavir 100 mg/d (only if PI naïve)	GI disturbances (mild) (take with a fatty meal, or up to 2 h after meal), headache, elevated transaminases, hyperglycaemia, dyslipidaemia
Atazanavir	PI	400 mg/d (only if PI naïve); or 300 mg with ritonavir 100 mg/d	Unconjugated hyperbilirubinaemia, hyperglycaemia, dyslipidaemia (low potential)
Fosamprenavir‡	PI	1 400 mg 12-hrly or 1 400 mg with ritonavir 200 mg/d	Rash, headache, GI upset, hyperglycaemia, dyslipidaemia
Iopinavir/ritonavir	Boosted PI	400/100 mg 12-hrly or 800/200 mg/d (only if PI naïve)	Asthenia, headache, GI upset, hyperglycaemia, dyslipidaemia

*Life-threatening reactions in bold type.

†Currently unavailable owing to contamination of manufacturing plant.

‡Limited availability in South Africa.