Potential Novel Approaches to Risk Identification in Advanced Peripheral Arterial Disease

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A thesis submitted to the Faculty of Health Science, University of the Witwatersrand for the degree of Doctor of Philosophy

2013

Abstract

Peripheral arterial disease (PAD) is a significant cause of morbidity and mortality in both economically developed and developing countries. Although the risk factors for PAD are well described, patients with PAD who develop critical lower limb ischaemia (CLI) are frequently asymptomatic prior to the development of CLI and the factors that determine outcomes in these patients are unclear. In the present thesis I therefore evaluated a number of potential novel risk approaches in patients with CLI, both for the development of CLI, as well as approaches that may better predict outcomes in CLI.

Atherosclerotic disease, the major pathophysiological process responsible for PAD, is now well recognized as causing an increased large artery pulse wave velocity (PWV) and central aortic pulse pressure (PPc). However, through the presence of arterial stenoses proximal to the femoral artery, carotid-femoral PWV may be reduced in advanced PAD. I therefore aimed to determine whether in the context of increases in central aortic pulse pressure (PPc), decreases in carotid-femoral pulse wave velocity (PWV) predicts the presence of advanced PAD. Applanation tonometry and vascular ultrasound were employed to assess carotid-femoral PWV, PPc and carotid intima-media thickness (IMT) in 1030 randomly selected healthy adults from a community sample and 217 patients with CLI. With adjustments for confounders, participants with CLI had an increased carotid IMT ($p<0.0001$) and PPc ($p<0.0001$), but a markedly reduced PWV (m/sec)(CLI=4.38±3.14, Community sample=6.78±2.47, $p<0.0001$). PWV was strongly correlated with PPc ($r=0.53$, $p<0.0001$) in the community sample, but not in CLI ($r=-0.04$). A stiffness mismatch index (PPc/PWV) showed increased values in participants with CLI over the full adult age range assessed. With carotid IMT, PPc or aortic augmentation index in the same regression model, an increase in the stiffness mismatch index (PPc/PWV) was independently associated with CLI ($p<0.0001$) and a PPc/PWV value>upper 95% confidence interval in the community sample strongly

predicted CLI (odds ratio=27.1, p<0.0001). In conclusion, in the context of an increased PPc, carotid-femoral PWV is markedly reduced in CLI. These results suggest that a stiffness mismatch index (PPc/PWV) may be a new risk marker for advanced PAD.

As infection with the human immunodeficiency virus (HIV) is common in South Africa, and this is increasingly translating into cardiovascular disease including CLI, it is important to be able to detect those HIV positive patients whom will develop CLI. Although ankle-brachial index may detect PAD, more general screening tools to detect those at risk of cardiovascular events are required. In this regard, carotid IMT measurements may be useful. The extent to which human HIV is associated with increases in IMT independent of conventional cardiovascular risk factors is unclear. Hence, I evaluated whether independent of conventional risk factors, an increased carotid IMT occurs in African HIV infected patients with chronic critical limb ischemia (CLI). Carotid IMT was measured in 217 sequentially recruited patients with CLI, 25 of whom were HIV positive and in 430 randomly selected controls from a community sample. As compared to HIV negative patients with CLI, HIV positive patients were younger (49±10 vs. 64±11 years, p<0.0001) and had a markedly lower prevalence of hypertension and diabetes mellitus (p<0.0001), but a similar proportion of patients smoked (76% vs. 67%). However, as compared to patients with CLI who were HIV negative, HIV positive patients had a similar increase in carotid IMT (HIV positive=0.75±0.14 mm; HIV negative=0.79±0.14 mm; Controls=0.64±0.15, p<0.0001 versus Controls) even after adjustments for age, sex and conventional risk factors (HIV positive=0.75.±0.13 mm; HIV negative=0.73±0.15 mm, Controls=0.66±0.15, p<0.005). IMT was similarly increased in HIV positive patients with CLI as compared to HIV negative patients with CLI when assessed in men, smokers, and black African patients only (p<0.05-0.0001), or in those who were receiving highly active antiretroviral therapy (n=12, 0.74±0.10 mm) as compared to those not receiving therapy (0.75±0.15 mm). As compared to controls, the age- sex- and conventional risk factor-adjusted odds of having an IMT≥0.8 mm was

similarly increased in patients with CLI who were HIV positive (odds ratio=8.89, CI=2.79-28.32, p=0.0002) as those who were HIV negative (odds ratio=2.70 CI=1.51-4.81, p<0.001). In conclusion, these results suggest that despite being of a younger age, with or without conventional risk factor adjustments, marked increases in carotid IMT in HIV is a risk factor for CLI. Thus, carotid IMT measurements may be a useful screening tool to detect those patients with HIV at risk of CLI.

Although asymptomatic decreases in left ventricular (LV) ejection fraction (EF) predict long-term mortality and decreased patency of endovascular interventions in patients undergoing vascular surgery, in patients with chronic critical lower limb ischemia (CLI), the prevalence of asymptomatic decreases in EF and the characteristic features thereof are unclear. I performed echocardiography in 93 sequentially recruited patients with CLI without symptoms of heart failure and 698 randomly recruited participants from a community sample. As compared to the community sample, patients with CLI had markedly reduced multivariate adjusted EF (CLI=56±12%, Community sample=67±11%, p<0.0001), LV midwall fractional shortening (FSmid)(p<0.0001), stroke volume index (SV)(p<0.0001), cardiac output index (CO)(p<0.05), and increased total peripheral resistance index (TPR)(p<0.05). In contrast to only 1/698 community participants, 26/93 (28%) patients with CLI had an EF<40%, of which only 5 had a previous myocardial infarction; and CLI was associated with a reduced EF independent of clinical evidence of coronary artery disease (CAD) and additional confounders (odds ratio=250, p<0.0001). In patients with CLI with an EF<40%, CO, SV and FSmid were all substantially reduced (p<0.0001), pro-brain natriuretic peptide concentrations and E/A were increased (p<0.05), whilst LV end diastolic volume index was marginally increased (p<0.05) as compared to those with an EF≥55%. Pro-brain natriuretic peptide had a poor sensitivity and specificity for the detection of an EF <40%. In conclusion, CLI is associated with a high prevalence of reduced EF independent of clinical evidence of heart failure, CAD and additional confounders, the main mechanism of which is a markedly reduced myocardial

systolic function. This translates into decreased CO and increased TPR, alterations that may contribute toward increased mortality or reduced patency of endovascular interventions after vascular surgery.

In conclusion, the results of this thesis suggest that longitudinal studies should be conducted to evaluate whether an arterial mismatch index (PPc/PWV) can predict the development of CLI independent of alternative cardiovascular risk factors; whether carotid IMT may be used to predict those at a high risk of cardiovascular events including CLI in HIV positive patients; and whether the presence of asymptomatic low LV EF may predict outcomes after surgery for CLI. Furthermore, the results of this thesis suggest that clarity is required to identify the exact large artery changes that characterize HIV positive patients with advanced PAD in South Africa.

DECLARATION

I declare that this thesis is my own unaided work except as indicated in the acknowledgements. It is being submitted for the degree of Doctor of Philosophy in the Faculty of Health Science, University of the Witwatersrand, Johannesburg. The work contained in this thesis has not been submitted for any degree or examination in this University or any other university.

Martin Brand ..... signed on the Second day of September 2013.

I certify that the studies contained in this thesis have been approved by the Committee for Research in Human Subjects of the University of the Witwatersrand, Johannesburg. The ethics approval number is M02-04-72 and renewed as M07-04-69.

Martin Brand ..... signed on the Second day of September 2013.

Prof. Gavin Norton (supervisor 1)	Prof. Martin Veller (supervisor 2)
Date:	Date: .....
.....	.....

PUBLICATIONS AND PRESENTATIONS ARISING FROM THE THESIS

Publication

1. Brand et al. *A Mismatch Between Aortic Pulse Pressure and Pulse Wave Velocity Predicts Advanced Peripheral Arterial Disease*. Eur J Vasc Surg epub 6 July 2013

2. Brand et al. *Carotid Intima-Media thickness in African patients with critical lower limb ischaemia infected with the Human Immunodeficiency Virus.* J AIDS Clinic Res 2012;3(7) 1-

Presentations

1. European Society for Surgical Research Congress 2013

Carotid intima media thickness in patients with chronic critical lower limb ischaemia infected with the HIV virus.

2. South African Surgery Research Society meeting 2012

- *Carotid intima media thickness in patients with chronic critical lower limb ischaemia infected with the HIV virus*. Received the Bunny Angorn Medal for the best research presentation
- *Left ventricular systolic dysfunction in chronic critical lower limb ischaemia*
- *Marked attenuation of age related increases in aortic pulse wave velocity in critical lower limb ischaemia*

3. Vascular Society of Southern Africa annual congress 2012

- *Marked attenuation of age related increases in aortic pulse wave velocity in critical lower limb ischaemia.* Won the best Junior researcher award.
- *Carotid intima media thickness in patients with chronic critical lower limb ischaemia infected with the HIV virus*.
- *Should primary health care not be incorporated into tertiary health care to prevent further mismanagement of patients in South Africa?*

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ACKNOWLEDGEMENTS

I declare that I designed the studies described in this thesis, in consultation with my supervisors. I collected all the clinical data from the patients with critical limb ischaemia with the assistance and supervision of an internationally certified echocardiographer, Sacha Grinter, who observed and analysed my echocardiographic data. The intima-media thickness images and readings were checked by Dr Muzi Maseko to ensure their accuracy.

First and foremost I would like to acknowledge Prof Gavin Norton for his time, patience, and continuous motivation in completing this thesis. Were it not for his energy and encouragement I would not have completed this work in the time allocated. The results of the various studies would not have been possible without access to his APOGH database, as well as to the equipment in his laboratory.

To Prof Martin Veller I would like to say thank you for keeping me grounded and focussed on the task at hand. Again, if it were not for his enthusiasm and support for the studies I would never have been able to complete the work in the allocated time either.

To Prof Angela Woodiwiss, under whose ever present supervision I performed all data analyses. Thank you for your unending patience, and constant willingness to help.

To the Carnegie Foundation for the Clinical Research Fellowship Award that allowed me to temporarily leave clinical practice to focus solely on my research, thank you.

To Prof's Pettifor (programme director), Kramer and Veriava, thank you for facilitating the Carnegie Fellowship at WITS, hopefully there will be many more.

To my wife, Liezl, thank you for understanding the path I chose to take, your constant support, and affection really made this an enjoyable journey. Thank you!

Finally, Joshua, who on several occasions during the first months of his life read papers or reread chapters late at night or early mornings while sleep bereft us both, thank you for all the smiles.

LIST OF ABBREVIATIONS

ABI	ankle-brachial index
ACEI	angiotensin converting enzyme inhibitor
AIx	augmentation index
AIc	central (aortic) augmentation index
AP	augmentation pressure
ART	anti-retroviral therapy
BK	below knee
BMI	body mass index
BNP	brain natriuretic protein
BP	blood pressure
CAD	coronary artery disease
CCA	common carotid artery
CD4 count	T-cell count, marker of cellular immunity
CHD	coronary heart disease
CI	confidence interval
CLI	critical limb ischaemia
cm	centimetre
CMV	cytomegalovirus
CO	cardiac output
CRP	C-reactive protein
cSP	central SBP
cPP	central PP
CVA	cerebrovascular accident
D	diameter of conduit artery (aorta) in water hammer equation

DP	diastolic blood pressure
DBP	diastolic blood pressure
DM	diabetes mellitus
E	Young's energy modulus of thin walled elastic tubes
E/A ratio	early (E) and late (atrial-A) transmitral velocity
ECG	electrocardiogram
EDV	end diastolic volume
EF	ejection fraction
ELISA	enzyme-linked immunosorbent assay
FSmid	left ventricular midwall fractional shortening
HAART	highly active anti-retroviral therapy
HbA_{1C}	glycated haemoglobin
HDL	high density lipoprotein
HF	heart failure
HIV	human immunodeficiency virus
hsCRP	highly sensitive C-reactive protein
IC	intermittent claudication
IMT	intima-media thickness
I-M complex	intima-media complex
IVS	interventricular septal wall thickness
kg	kilogram
kg/m^2	kg per meter^2
LDL	low density lipoprotein
LV	left ventricle
LVED	LV end diastolic
LVEDD	LV end diastolic diameter

LVEDV	LV end diastolic volume
LVESD	LV end systolic diameter
LVESV	LV end systolic volume
LVID	LV internal diameter
LVH	LV hypertrophy
LVM	LV mass
LVMI	LVM indexed for body surface area
MAP	mean arterial pressure
mg/l	milligrams per litre
MHz	megahertz
MI	myocardial infarction
ml	millilitres
mls/m^2	millilitres per square meter
mm	millimetre
M-mode	one dimensional echocardiogram
mm Hg	millimetres of mercury
mmol	millimole
mmol/day	millimoles per day
MP	mean blood pressure
msec	milliseconds
m/sec	meters per second
mV	millivolts
n	number (sample size)
NM	not mentioned
NT-proBNP	N-terminal pro-brain natriuretic peptide
OR	odds ratio

p	fluid density in conduit artery (aorta)
P1	central (aortic) forward component pressure
p value	probability value
PAD	peripheral arterial disease
pg/ml	pictogram per millilitre
PP	pulse pressure
PPc	central (aortic) PP
PPp	peripheral (brachial) PP
PPc/PWV	stiffness mismatch index
proBNP	pro-brain natriuretic peptide
PTA	percutaneous transluminal angioplasty
PWT	posterior wall thickness
PWV	pulse wave velocity
r	correlation coefficient
RA	rheumatoid arthritis
RWT	relative wall thickness
SAS	statistical analyses software
SBP	systolic blood pressure
SBPc	central SBP
SD	standard deviation
SEM	standard error of the mean
SLE	systemic lupus erythromatosis
SOWETO	South Western Township (Johannesburg, South Africa)
SP	Systolic blood pressure
SV	stroke volume
SV_t	stroke volume calculated using the Teichholz method

TG	triglyceride
TPR	total peripheral resistance
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

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Preface

A high proportion of patients with peripheral arterial disease (PAD) only become symptomatic with the onset of chronic critical limb ischaemia (CLI), and among these, there is a high rate of limb loss and mortality. The ability to identify those patients who will develop CLI, and to predict their clinical outcomes requires considerable refinement. The present thesis was therefore prompted by a need to identify novel risk factors both for the development of CLI, as well as for predicting the outcomes in patients CLI. What are the hypotheses tested in the present thesis?

Although an increased aortic stiffness, as indexed by carotid-femoral pulse wave velocity (PWV) is associated with cardiovascular disease, in the present thesis I hypothesized that through stenoses of arteries proximal to the femoral artery, that in CLI, PWV may be attenuated, whilst central aortic pulse pressure (PPc), an alternative index of aortic stiffness remains increased. Using this approach I tested the hypothesis that PPc/PWV, which I have termed a stiffness mismatch index, may be significantly increased in patients with CLI independent of alternative indices of large artery disease.

Human immunodeficiency virus (HIV) is a risk factor for occlusive arterial disease including CLI. However, how best to identify that HIV positive patient at risk for CLI is unknown. In the present thesis I therefore tested the hypothesis that through the vascular effects of HIV, carotid intima-media thickness (IMT), usually considered an index of systemic atherosclerosis, may be markedly increased in patients with CLI whom are HIV positive, despite considerably lower levels of cardiovascular risk factors.

As reductions in left ventricular systolic function (EF) predict outcomes in patients with symptoms of heart failure undergoing surgery for PAD (increased mortality and reduced stent patency), in the present thesis I also explored the hypothesis that a significant proportion of patients with CLI may have a reduced EF and no symptoms of heart failure, and that these decreases in EF may be accompanied by haemodynamic changes that could contribute toward

mortality and a reduced stent patency. I therefore assessed the possibility that in patients with CLI without clinical evidence of heart failure, that identifying the presence of systolic dysfunction prior to surgery may assist in risk stratifying patients at risk of poor outcomes. These hypotheses were tested in order to evaluate whether a prospective study was worth conducting.

This thesis is written as a series of semi-independent chapters, each with its own introduction, methods, results and discussion section. The thesis begins with a review chapter that highlights the current understanding and controversies in the field, and leads the reader through a series of arguments in support of conducting the studies described in the present thesis. The thesis concludes with a summary chapter, which consolidates the findings of each chapter and underscores the novelty of the findings by placing the studies in the context of our present understanding of the field. The data provided in chapter 2 has been published in the European Journal of Surgery, and the data in chapter 3 has been published in the Journal of AIDS and Clinical Research, the data in chapter 4 are currently in preparation for submission to an international journal for review.

CHAPTER 1

Potential Novel Approaches to Risk Identification in Advanced Peripheral Arterial Disease: Is There a Need and Which Measures Could be Considered?

1.1 Introduction

In this thesis I explored a number of potential novel approaches to risk assessment in advanced peripheral arterial disease (PAD). In this chapter I provide a series of arguments in favour of these approaches, highlighting the evidence that led me to evaluate the novel approaches studied. However, before presenting the arguments, I will define PAD and what is meant by advanced PAD, and emphasise the importance of studying PAD in general as well as the need for identifying novel approaches to risk assessment in advanced as opposed to early PAD. I will subsequently develop the arguments in favour of exploring novel approaches to risk assessment in advanced PAD in the context of current approaches to risk assessment. Thereafter I will describe the evidence that led me to generate the hypotheses that substantiate the aims of the thesis in the context of the hypotheses generated.

1.1.2 Definition and prevalence of peripheral arterial disease

Peripheral arterial disease is characterized by blocked arteries in the limbs and is manifested by ischaemic pain induced by exertion as a direct consequence of an inability to adequately perfuse the lower limbs and is relieved by rest. This symptom is called intermittent claudication (IC), clinically there are absent or markedly diminished pulses on physical examination. Claudication generally results from a single level of arterial stenosis or occlusion, the most common cause of which is advanced atherosclerosis (Ross 1979), resulting in inadequate arterial perfusion to meet the demands of skeletal muscle metabolism. The presence of inadequate perfusion of the lower extremities can be objectively measured using a number of approaches. The simplest technique is to identify an abnormal ankle-brachial index (ABI) where blood pressure (BP) measured at the ankle is expressed as a proportion of BP measured at the brachial artery (Pasternak *et al*. 2004). To identify the

presence of PAD, an ankle brachial index < 0.90 is 90% sensitive and 95% specific for PAD (Greenland *et al.* 2000).

In economically developed countries, few studies have been published from developing countries, the incidence of asymptomatic PAD is high. Using ABI to identify PAD in a general population, the prevalence of PAD ranges from 3% to 10%, increasing to 20% in persons over 70 years of age (Mahoney *et al.* 2010, Leng *et al.* 1996). The prevalence of IC increases from 3% in persons aged 40 years to 6% in those aged 60 years or more (Makowsky *et al.* 2011). A European study conducted in 7715 participants ≥ 55 years of age noted an incidence of asymptomatic and symptomatic PAD in 19.1% and 1.6% of participants respectively (Meijer *et al.* 1998). A more recent study conducted in the United States of America (USA) indicated that asymptomatic PAD may occur in 4.5% of those over 40 years of age and 14.5% of those over 70 years of age, with the highest prevalence rates noted in non-Hispanic blacks (Selvin *et al.* 2004). The rate of progression of patients from asymptomatic to symptomatic PAD is not known, as no longitudinal studies have been published in asymptomatic patients with PAD, however it is considered to be low (Norgren *et al.* 2007).

There are few data on the prevalence and incidence of PAD from economically developing countries. However, in a cross-sectional study conducted in 1262 participants (mean age of 46±15 years) in India, the prevalence of symptomatic PAD was 3.2% (Premalatha *et al.* 2000). Although there are no studies to indicate the extent of the burden of PAD in South Africa, the high prevalence of traditional risk factors for cardiovascular disease (Thorogood *et al*. 2007, Tollman *et al*. 2008, Mash *et al*. 2012, Bradshaw *et al.* 2007, Heunis *et al.* 2006) suggests that a similarly high proportion of South Africans either suffer from or will develop PAD as that noted in developed countries.

Importantly, as indicated in the preceding discussion, racial disparities exist in the incidence of PAD (Selvin *et al*. 2004). Indeed, as compared to Caucasian Americans, African

American's have an increased rate of PAD (Zheng *et al*. 1997) and hence it is possible that in countries such as South Africa, where the majority of the population are of black African ancestry, even higher prevalence rates of PAD than that noted in developed countries may occur. Although groups of African ancestry have a higher prevalence of traditional risk factors for PAD, the aforementioned ethnic disparity remains following adjustments for traditional risk factors (Criqui *et al.* 2005). Novel serum risk markers do not explain the higher incidence of PAD in groups of African ancestry (Ix *et al*. 2008).

1.1.3 How does peripheral arterial disease translate into a burden of disease?

Epidemiological studies have provided ample evidence to show that disease in one vascular bed is associated with disease in other vascular beds (Mautner *et al.* 1992, Salonen *et al.* 1991, Newman *et al.* 1993). Moreover, the presence of disease in any vascular bed is associated with an increased incidence of overall cardiovascular events and mortality (Criqui *et al*. 1992, Nadelmann *et al.* 1990, Newman *et al.* 1993). In this regard PAD is no exception, and is often associated with comorbidities produced by atherosclerosis in alternative vascular beds. In a necropsy study of patients with previous lower limb amputation as a result of PAD, 92% of these patients had atherosclerotic plaques in their coronary vessels causing a tight stenosis (>75% reduction of luminal diameter) or occlusion of these vessels (Mautner *et al.* 1992). Moreover, a prospective registry demonstrated that 11.6% of patients with IC will die either from a myocardial infarction or stroke within two years of diagnosis of their IC (Stansby *et al*. 2011). The presence of PAD is associated with an increased cardiovascular mortality as compared to people without PAD (Mahoney *et al*. 2010, Criqui *et al.* 1992). Concomitant cerebrovascular and coronary artery disease may account for mortality rates of 13.4% at 6 months after surgery for PAD, 19-25% at 12 months (Bertele *et al.* 1999, Varty *et al.* 1998) and 67% at 5 years (Dorros *et al*. 2001). In an observational study an ABI < 0.90

was noted to independently predict cardiovascular morbidity and mortality (Mohler 2003), and there is a stepwise increase in cardiovascular morbidity and mortality as ABI decreases (Newman *et al*. 1993). Involvement of multiple segments of the arterial tree supplying the lower limb predicts the risk for death. Patients with unisegment disease have a relative risk for cardiovascular death that is twice normal as compared to a risk that is 7 times normal in those with multisegment disease (Vogt *et al*. 1993).

There is no doubt that the presence of PAD, diagnosed either clinically or according to ABI values, predicts cardiovascular outcomes. A significant question is the extent to which PAD ultimately translates into an ischaemic limb that will either require revascularisation, or results in limb loss, or causes death.

1.1.4 Consequences of peripheral arterial disease for the lower limb

The end stage of PAD is chronic critical lower limb ischaemia (CLI)(Varu *et al.* 2010) and is defined as ischaemic pain at rest for more than two weeks, or the presence of ulcers or gangrene attributable to occlusive arterial disease (Rutherford *et al.* 1997). The clinical diagnosis of CLI is objectively confirmed by an ankle pressure less than 50 mm Hg, or a toe pressure less than 30 mm Hg, or an ABI less than 0.40 (Norgren *et al*. 2007). There are approximately 500 to 1000 new cases of CLI per million of the population every year in Europe or North American (Kannel *et al.* 1970).

The interventions usually required to manage CLI include revascularisation procedures (either endovascular or open vascular surgery) or amputation. Critical limb ischaemia is associated with a high risk of limb loss in the absence of a revascularization procedure (Varu *et al*. 2010). The amputation rate after 12 months of follow-up in a series of 142 patients (169 limbs) with CLI who did not undergo revascularization was 15% limbs with an ABI of 0.5-0.7, 32% limbs with an ABI < 0.5, and 43% for limbs with an ABI < 0.4

(Marston *et al.* 2006). Observational studies of patients diagnosed with CLI reveal that at 1 year, 50% of patients will remain amputation-free, although they may still be symptomatic, 25% will require a major amputation and the remaining 25% will have died (Norgren et al. 2007). On the other hand a two year prospective study of claudicants in the United Kingdom showed that 8.4% of these patients had died and 11.6% had suffered either a myocardial infarction or a cerebrovascular event (Stansby *et al*. 2011). In other words, symptomatic PAD is a significant marker for adverse cardiovascular events, and the incidence of these events increases with increasing severity of PAD.

Symptomatic PAD is not only a significant disease for the patient, but also a burden to society through a loss of life and limb with the generation of dependent individuals unable to contribute to the workforce in many instances, and a major burden to healthcare budgets. In this regard, 40.3% of claudicants require at least one hospital admission, and 17.4% require a revascularisation procedure (Stansby *et al*. 2011). In the USA, the number of patients undergoing a percutaneous transluminal angioplasty (PTA) had increased threefold from 1999 to 2007, whilst open bypass grafts had decreased by a third, despite the cheaper costs of an open vascular procedure ($22 910 vs $23 196) (Sachs *et al*. 2011).

1.1.5 Is the transition from intermittent claudication to critical lower limb ischaemia clearly identifiable?

Intuitively one would expect IC to be a strong risk factor for the development of CLI. Some studies show that 20-30% of claudicants will eventually require a revascularization procedure (McCaslin *et al.* 2007, Mahoney *et al.* 2010). However only 2% of claudicants will develop CLI (Hirsch *et al*. 2006). Moreover, in a prospective multi-centre study in patients requiring an amputation due to irreversible ischaemia resulting from CLI, 55% of these

patients were asymptomatic (no claudication) in the six months prior to their amputation (Dormandy *et al*. 1994). Hence IC is not an obligate risk factor for CLI.

The question therefore remains as how best to identify asymptomatic persons at risk for CLI? Although ABI measurements provide some degree of ability to identify patients with CLI who will require amputation (Marston *et al*. 2006), this approach does not allow for the identification of those who are likely to develop CLI. There is therefore a need for better, simple, reliable and easy to perform tools which may detect either the patient diagnosed with PAD who has sufficiently severe atheromatous disease that it will progress to CLI, or tools that can be employed in the asymptomatic high risk patient that will suggest that their atheromatous disease is sufficiently advanced that CLI may occur.

1.1.6 Can one adequately predict perioperative outcomes in patients with critical lower limb ischaemia?

Patients with PAD require a significant number of hospital admissions for revascularisation procedures (Spittell 1990). For every 1000 symptomatic patients with PAD, 99.8 will require a PTA, 58.3 will require an open vascular procedure, and 31.8 will require a lower limb amputation (Mahoney *et al.* 2010). Moreover, for every 1000 asymptomatic patients with PAD, 59.7 will require a PTA, none will require an open vascular procedure, and 7.5 will require a lower limb amputation (Mahoney *et al.* 2010). With the high rate of surgery required for PAD, it is important to consider all outcomes in such a high-risk group of patients. In a large multicenter trial 16% of patients with PAD requiring surgery had repeat procedures only to eventually die or sustain limb loss within 12 months following the procedure (Adam *et al.* 2005). All patients with CLI require a revascularisation procedure (Norgren *et al.* 2007). Of these patients 51% will eventually require an amputation, 32% of which will be a major lower limb amputation, and only 26% of CLI patients will survive for

another 5 years (Jamsen *et al.* 2002). Thus, even with the best available care, outcomes following surgery are poor. The currently accepted predictors of a poor post-intervention outcome and a diminished survival include the extent of the arterial disease below the knee, an increased body mass index, older age, smoking, serum creatinine concentrations, a previous myocardial infarct or cerebrovascular event, and congestive cardiac failure (Meltzer et al. 2012, O'Brienn-Irr *et al.* 201, Bradbury et al. 2010).

With respect to the incidence of heart failure in PAD, heart failure is much more common in patients with symptomatic PAD when compared to a general population. Approximately 8% of patients with symptomatic PAD may have heart failure, and symptomatic PAD doubles the risk for developing heart failure (Anand *et al.* 2007). Furthermore, in patients with CLI, as many as 34% may have heart failure (O'Brian-Irr *et al.* 2011). The higher incidence of heart failure in patients with PAD may largely be explained by a higher incidence of coronary artery disease that accompanies PAD (Norgern *et al.* 2007). Indeed, in patients with CLI, as many as 21% may have had a myocardial infarction up to 3 months prior to developing limb ischaemia (O'Brien-Irr *et al.* 2011).

Heart failure is strongly associated with an increased peri-operative mortality and morbidity in major non-cardiac surgery (Rhode *et al.* 2001), as well as in patients requiring infra-inguinal arterial reconstruction for PAD (Meltzer *et al.* 2012, Shrikhande *et al.* 2007). In patients with symptomatic PAD requiring an operative procedure, the perioperative mortality rate is significantly higher in those with as compared to those without heart failure (Meltzer *et al.* 2012). This is not surprising considering the fact that despite a number of interventions developed over the past half a century for the treatment of heart failure, heart failure persists to be associated with a high mortality rate (Hunt *et al.* 2009). The higher perioperative mortality rate in in patients with PAD undergoing surgery has resulted in the practice of offering patients with symptomatic heart failure an endovascular procedure (angioplasty/

stent) whenever possible. However, the patency of endovascular interventions may be compromised by the presence of heart failure.

Heart failure associated with left ventricular (LV) systolic dysfunction, defined by an LV ejection fraction (EF) <40%, has recently been shown to be an independent risk factor for loss of stent patency after one year follow-up (Meltzer *et al*. 2012). There may be several explanations for this. Patients with heart failure are often characterised by vasoconstriction (Zelis *et al.* 1968), thus increasing peripheral resistance in an attempt to maintain vital organ perfusion (Ledoux *et al*. 2003, Kubo *et al.* 1991). The increased peripheral resistance may reduce peripheral blood flow and hence produce stasis. Moreover, LV systolic dysfunction may be associated with a reduced overall flow (cardiac output) (Dickstein *et al*. 2008) thus resulting in a poor perfusion of the peripheral vasculature. With stasis ans a low flow rate, an increased chance of coagulation is obviously the consequence.

Although the presence of clinical signs of heart failure and associated evidence of coronary artery disease are risk factors for a poor outcome in PAD, as will be emphasised in the subsequent discussion, it is also possible that asymptomatic cardiac disease may predict outcomes. In other words, there may be a significant number of patients with PAD that have concomitant subclinical evidence of cardiac pathology PAD, and consequently will be at risk for a poor perioperative outcome.

1.1.7 Possible novel approaches to predicting risk in advanced peripheral arterial disease were evaluated in this thesis

As indicated in the aforementioned discussion, there is a need to identify better simple and easy to perform tools which may detect either patients with PAD who have sufficiently severe atheromatous disease that it will progress to CLI, or tools that can be employed in screening asymptomatic high risk patients that will suggest that their atheromatous disease is

sufficiently advanced that CLI may occur. There is also a need to identify patients who are at risk of poor perioperative outcomes. In this thesis I explored a number of possible novel approaches to assessing risk in advanced PAD. In the remaining sections of this chapter I will discuss current approaches that are used to detect advanced PAD, as well as risk assessment for poor outcomes in the perioperative period in patiensts with advanced PAD. This will provide the background to the novel approaches that I explored.

1.2 Current risk assessments for peripheral arterial disease

Advances in our understanding of the pathogenesis of atherosclerotic vascular disease gave rise to the concept of cardiovascular risk factors (Kannel *et al.* 1961). Risk factor assessment, which involves evaluating global risk related to a combination of risk factors that include age, sex, BP, blood cholesterol concentrations, smoking and diabetes mellitus, is important to accurately guide primary and secondary prevention strategies. A component of many risk factors is hereditary (and therefore not necessarily modifiable), but there is always an acquired component related to behavioral and environmental factors which is potentially amenable to manipulation. Risk factor assessment to quantify components contributing to the future risk of cardiovascular events was developed during the Framingham study (Wilson *et al.* 1998) and has been substantiated by numerous subsequent studies, too numerous to cite.

What is our understanding of the contribution of the most important modifiable traditional risk factors toward PAD? Traditional risk factors for PAD are well defined and include diabetes mellitus, smoking, increasing age, hypertension and dyslipidaemia (including increased low-density lipoprotein cholesterol levels and decreased high density lipoprotein levels) (Murabito *et al.* 1997). In this thesis I evaluated the possibility that risk evaluation beyond these traditional cardiovascular risk factors may be used to identify patients at risk of advanced PAD. However, I will first explain to what extent these risk factors contribute

toward PAD. This is not a primary focus of this thesis and hence this topic will only be dealt with at a cursory level, to the extent that it provides a necessary background to the studies conducted.

1.2.1 Hypertension and peripheral arterial disease

Hypertension currently affects one billion individuals globally and contributes to 62% of cerebrovascular disease (strokes); to 49% of coronary heart disease (myocardial infarctions); and to 65% of PAD (World Health Organisation 2003). Its prevalence is increasing in both economically developed and developing countries (World Health Organisation 2003, Chobanian 2007). Estimates of the prevalence of hypertension range from 28% in North America to 44% in some European countries (Wolf-Maier *et al.* 2003). In South Africa hypertension affects approximately 26% of the entire population, (Steyn *et al.* 2008) with an estimated 40 to 50% of urbanised populations of African descent being hypertensive (Malhotra *et al.* 2008, Maseko *et al*. 2010). In these urban communities hypertension contributes to the development of 50% of strokes (Norman *et al*. 2007), 42% of myocardial infarctions (Steyn *et al.* 2005, Norman *et al.* 2007) and 33% of heart failure (Stewart *et al.* 2008, Connor *et al.* 2009). Presently there are no data to indicate the extent to which hypertension contributes toward PAD in economically developing populations. As cardiovascular disease is the leading causes of death in the elderly and the third most common cause of death in younger African age groups (Tollman *et al*. 2008), hypertension clearly accounts for a significant burden of disease in groups of African descent living in developing countries. Management of hypertension has become a global priority (Zarcostas 2010). However despite growing primary health care programmes therapeutic goals are still not being achieved, including South Africa (Heunis *et al.* 2006).

Hypertension in general is managed poorly. In the United States of America only 34-35% of all hypertensives and 55% of intensively treated hypertensives achieve their target BP

level (Hertz *et al*. 2005, Cutler *et al*. 2008). The control of BP in some European countries may be far worse (Wolf-Maier *et al*. 2004, Wang *et al*. 2007). Strikingly in South Africa, where the majority of the population are of black African ancestry, only 14% of all hypertensives (Steyn *et al*. 2001) and 33-44% of hypertensives attending primary health care clinics (Dennison *et al*. 2007, Steyn *et al*. 2008) achieve adequate BP control. Furthermore, in urban communities of African descent, 46% may have hypertension of which more than half may not receive antihypertensive therapy (Maseko *et al*. 2010) and of those receiving treatment only 36% achieve target BP values (Maseko *et al*. 2010). Thus, globally and particularly in groups of African descent, hypertension is poorly controlled.

1.2.2 Diabetes mellitus and peripheral arterial disease

The global incidence of diabetes mellitus was 135 million in 1995, and is expected to reach 300 million by 2025, with the greatest increase occurring in Africa and Asia (King *et al.* 1998). The mortality rate of insulin-dependent diabetes in sub-Saharan Africa within 5 years of being diagnosed is 41% (Hall *et al*. 2011). Gangrene and systemic sepsis originating from diabetic foot ulcers are a common cause of death (Abbas *et al*. 2007). One of the principal pathophysiological mechanisms that contributes toward the development of a "diabetic foot" is PAD (Jude *et al*. 2001). Diabetic patients with PAD have more advanced PAD and worse outcomes than non-diabetic patients with PAD, and their outcome is augmented with poorer glucose control (Jude *et al*. 2001). Diabetes care in South Africa is poor. In a rural community only 15.7% of diabetics achieved their therapeutic target, with a mean HbA_{1c} of 11.3% (Rotchford *et al*. 2002) and 49.4% of diabetics attending tertiary hospital affiliated clinics achieve their therapeutic target (Levitt *et al*. 1997).

1.2.3 Dyslipidaemia and peripheral arterial disease.

Many epidemiological studies have demonstrated that elevated total serum cholesterol concentrations increase the risk for cardiovascular events, however this excessive risk only

occurs in patients who are already at an increased risk of cardiovascular events (Taylor *et al.* 2011). Counter intuitively treating patients with an elevated circulating cholesterol concentration with no other risk factors may be harmful (Redberg *et al.* 2012). Although undoubtedly dyslipidaemias are likely to contribute substantially to PAD (Fowkes *et al.* 1992), presently there is little evidence to suggest the extent to which this occurs as most longitudinal studies report on the role of lipid levels as risk factors for the development of strokes and myocardial infarcts (which occur much more frequently than symptomatic PAD) as opposed to symptomatic PAD and CLI. However, treatment with statins regardless of a patient's presenting serum cholersterol level results in a 24% reduction of the first adverse cardiovascular event (stroke/ MI) as well as peripheral vascular events in patients with PAD. (Heart Protection Study Collaborative Group 2007). Furthermore statin therapy has been shown to be cost-effective due to the avoidance of cardiovascular events and the treatment there of in PAD (Mihaylova *et al.* 2005).

1.2.4 Cardiovascular risk beyond traditional risk factors.

While the use of global traditional risk factor assessment was a conceptual advance with proven clinical utility, traditional risk factors such as age, sex, BP, blood cholesterol concentrations, smoking and diabetes mellitus (or glucose intolerance) only account for less than half the risk of cardiovascular events (Gordon *et al.*1974). The concept of the extent to which traditional risk factors explain the burden of cardiovascular disease is somewhat contentious. Some epidemiological studies have shown that traditional risk factors do not account for differences in cardiovascular mortality across socio-economic groups (Harald *et al.* 2008), while others have shown that there is a very good correlation between these risk factors and cardiovascular events (Menotti *et al.* 2009). Moreover, patients on adequate treatment for recognised risk factors still experience adverse cardiovascular events (Kohro *et*

al. 2008, Szecsenyi *et al.* 2008). Indeed, prospective registries show that 11.6% of patients with IC will die from a myocardial infarction or stroke within two years of diagnosis, despite appropriate treatment of their risk factors (Stansby *et al.* 2011). What are some of the alternative possible risk factors that may account for cardiovascular disease?

Accelerated atherosclerosis is a feature of inflammatory vascular disorders and inflammation is a recognised risk factor for cardiovascular disease. Indeed, the incidence of coronary artery disease is 50 times greater in those with systemic lupus erythromatosis (SLE), than in patients without SLE (Manzi *et al.* 1997) and traditional risk factors do not account for this marked contrast in the prevalence of cardiovascular disease (Esdaile *et al.* 2001). Rheumatoid arthritis (RA), which is similarly a disease associated with systemic inflammation, is associated with a two times greater risk of cardiovascular mortality, independent of traditional risk factors (Luqmani *et al.* 2009). There is also significant evidence to suggest that increases in inflammatory markers at a population level or in high-risk patients without obvious evidence of inflammatory disease (such as SLE or RA) contributes toward cardiovascular disease. In this regard circulating high-sensitivity C-reactive protein (hs-CRP) concentrations are associated with an increasing prevalence of thin-cap atheromatous plaques that are prone to rupture, as opposed to calcified plaques, as well as to a greater risk of sudden death (Burke 2002). Moreover, hs-CRP concentrations correlate with total plaque volume in the coronary vasculature (Rubin *et al.* 2011, Burke *et al.* 2002) and in a systematic review, the risk of stroke, myocardial infarction and cardiovascular deaths was noted to increase with increasing circulating hs-CRP concentrations (Kaptoge *et al.* 2010). In this regard, the risk for stroke or myocardial infarction related to circulating hs-CRP concentrations was comparable with that of the risk attributed to traditional cardiovascular risk factors (Kaptoge *et al.* 2010). Although, the role of hs-CRP in cardiovascular disease is not supported by all studies (Hunt *et al.* 2011, Folsom *et al.* 2001, Redberg *et al.* 2000, Tracy

et al. 1997) hs-CRP concentrations are nevertheless being used in cardiology practices as a screening tool to identify the high-risk patient (Ridker *et al.* 2007).

However, systemic inflammation alone cannot explain the excess burden of cardiovascular disease beyond traditional risk factors. Indeed, PAD is a condition characterised by a low level chronic inflammatory state (Ross *et al.* 1993), and yet PAD is still only partially explained by traditional risk factors (Khawaja *et al.* 2009). Thus, the use of traditional cardiovascular risk factor assessments, even when used in combination with measures of circulating inflammatory markers, may be insufficient to identify all of the risk associated with cardiovascular disease. What are some alternative novel, easy to use, and relatively inexpensive approaches that may improve risk prediction beyond traditional risk factors?

1.3 Recent novel, relatively inexpensive, and easy to use approaches to predicting cardiovascular risk.

An important advance in risk factor assessment has been the principle that prior to the development of a cardiovascular event, cardiovascular target organ damage may be detected using a variety of approaches. The hypothesis in this regard is that traditional risk factor assessment does not account for temporal effects of risk factors on the cardiovascular system. By assessing organ damage however, one may evaluate an accrual of effects of risk factors over time. This concept has evolved to the point that current guidelines recognise the identification of organ damage as part of risk prediction. A number of measures of target organ damage have been shown to predict cardiovascular outcomes beyond traditional cardiovascular risk factors and these include electrocardiographic or echocardiographic evidence of left ventricular hypertrophy, a reduced estimated glomerular filtration rate, increases in urinary microalbumin-to-creatinine ratios, indices of left ventricular dysfunction,

and measures of abnormalities in large vessel structure and function. More recent studies advocate the use of even more advanced techniques. A review of all of these measures goes beyond the scope of this thesis. However, I explored novel approaches to the use of measures of large vessel structure (carotid intima-media thickness) and function (indexes of aortic stiffness) and left ventricular function as possible risk markers in advanced PAD. Hence in the present section I will briefly describe our understanding of the current use of these measures as risk predictors and consequently in subsequent sections (section 4.0) I will reinforce my hypotheses for exploring the novel use of these risk markers in advanced PAD.

1.3.1 Indices of aortic stiffness

The stiffness of large vessels such as the aorta, is determined by a number of cellular and interstitial constituents of the media. The media consists of elastic lamellae (which determines the elasticity of large vessels) with intervening layers of smooth muscle cells, collagen fibers (which are generally thought to determine an increased stiffness) and ground substance. The distribution of elastin and collagen differs markedly along the longitudinal axis of the aorta (Dingemans *et al*. 2000). The collagen-to-elastin ratio reverses from the elastin-dominant proximal aorta to the collagen dominant peripheral large arteries (Dingemans *et al.* 2000). Although both elastin and collagen have a very low turnover rate (Fleischmajer *et al*. 1990, Johnson *et al*. 1995), the characteristics and concentrations of these substances in large vessels may vary considerably over time. A major determinant of the rigidity of the arterial wall are the atherosclerotic processes that accompany aging, as well as a number of cardiovascular risk factors. In this regard, aging is associated with a loss of elastin, but not collagen (Benetos *et al*. 1993). Hypertension, diabetes mellitus, dyslipdaemia and smoking may either increase smooth muscle cell size and number, decrease elastin concentrations, increase collagen concentrations, or modify the cross-linked properties of

collagen, rendering a stiffer vascular wall (Summer *et al.* 1969, Roberst *et al.* 1973, Ross *et al.* 1986, O'Rourke *et al.* 2002). As a consequence, a number of groups have sought to determine whether alterations in measures of large artery stiffness predict cardiovascular outcomes.

As a consequence of an increased large artery stiffness, whilst diastolic BP either remains unchanged (or may even decrease), systolic BP increases for a number of reasons, including the effect of a decreased distensibility of the ascending aorta during ventricular ejection as well as possibly through increases in wave reflection along stiffer large arteries (which contribute toward an enhanced degree of aortic systolic pressure augmentation). The consequence is an increased central aortic pulse pressure (PPc) which is transmited to the periphery as peripheral or brachial artery pulse pressure (PPp). Brachial artery PPp is therefore thought to reflect changes in arterial stiffness (Safar et al 2003). Peripheral (PP) was therefore considered as an independent marker for cardiovascular disease (Franklin et al 1999). However, it is now recognised that brachial artery BP considerably overestimates systolic BP and PPc (Pauca *et al.* 1992). This is in part due to altered peripheral wave reflection (Hamilton 1944) as well as wave distortion (Latham *et al.* 1985). Hence more direct measures of arterial stiffness or PPc have been sought. In this regard, the speed of wave travel increases with an increased large vessels stiffness, and this has resulted in the use of aortic pulse wave velocity (PWV) as one more direct measure of aortic stiffness (see Figure 1.1). Second, PPc may be determined non-invasively using easy and reproducible measurements employing applanation tonometry (see Figure 1.2) at the radial artery and a generalised transfer function to derive a central aortic waveform, or through carotid tonometry which may not require a transform function. Moreover, from these central aortic waveforms, the augmented pressure wave can be identified and expressed as a proportion of aortic pulse pressure (augmentation index-AIx), which is in part determined by stiffer arteries. Hence AIx is also employed as a measure of aortic stiffness (Laurent *et al.* 2006).

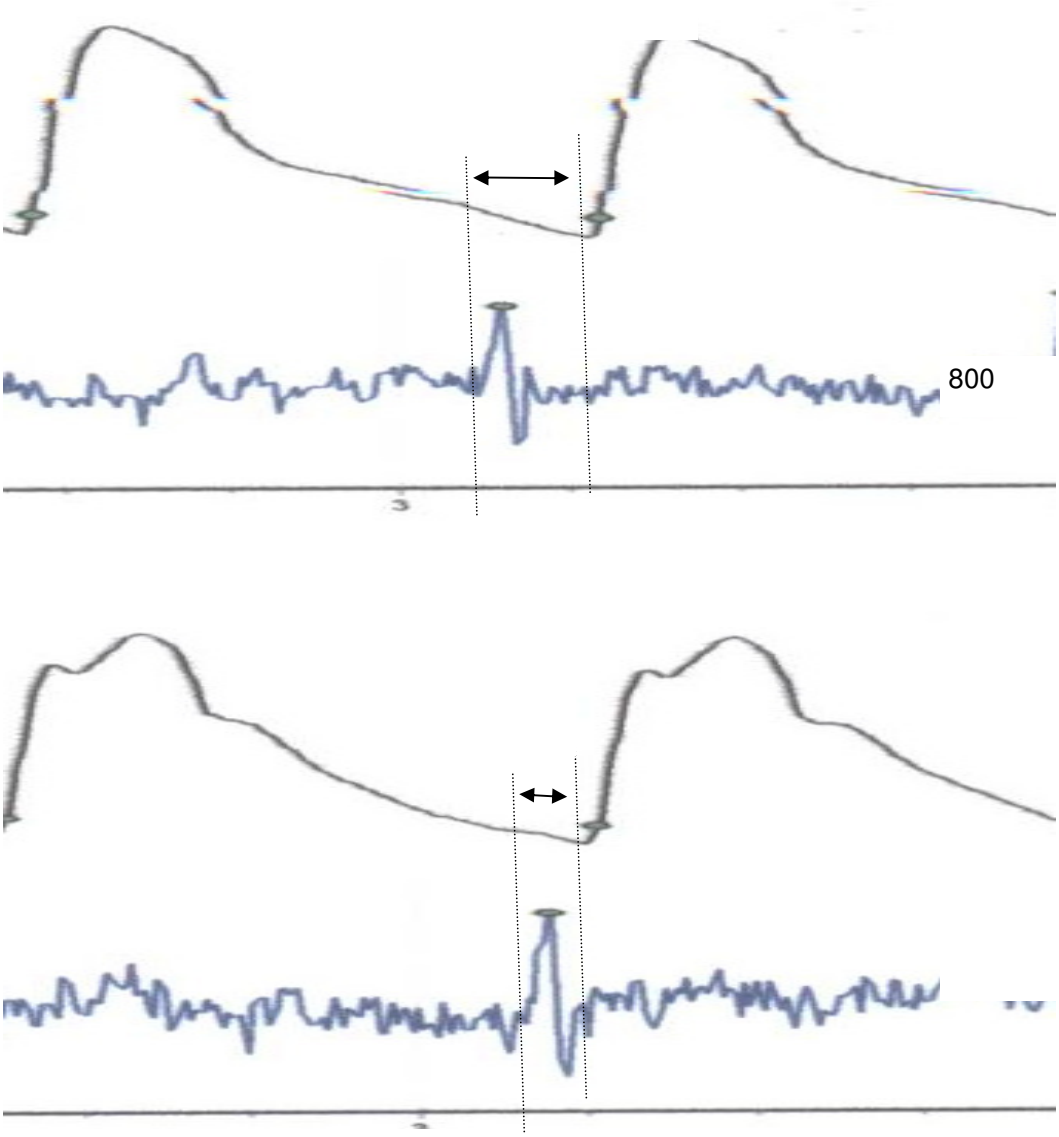

Figure 1.1. Examples of femoral and carotid artery pulse waves obtained using applanation tonometry from the same participants.

Together with simultaneous electrocardiographic recordings aortic pulse wave velocity is calculated. The arrows indicate the time between electrical events and the arterial pressure changes in the carotid and femoral arteries used to calculate PWV.

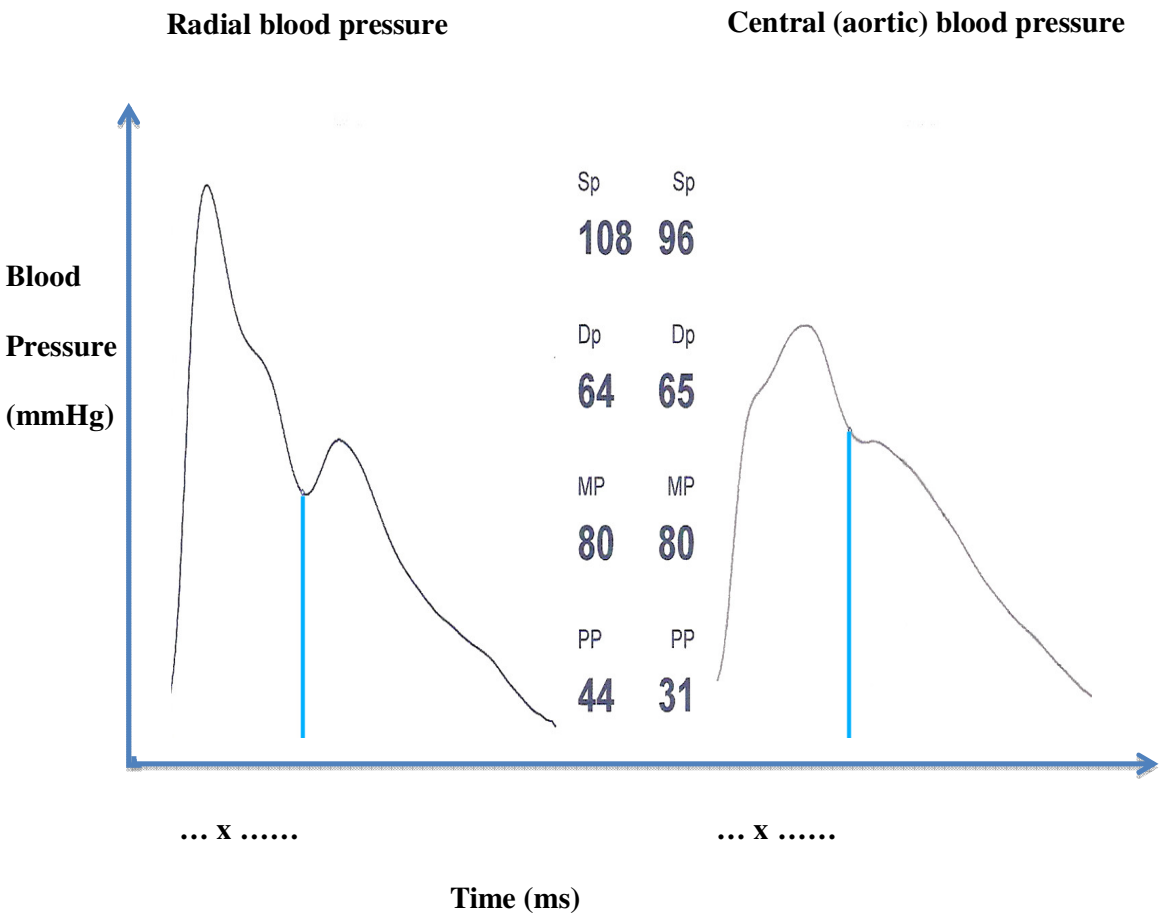

Figure 1.2. Examples of a pulse wave recording obtained to determine central haemodynamics.

The figure shows the radial artery pulse wave obtained from applanation tonometry (left panel) and the aortic pulse wave derived from a population-based transfer function built into the software (right panel). The first and second systolic shoulders are identitfied, separated by the same time gap 'x'. Sp, systolic pressure; Dp, diastolic pressure; MP, mean blood pressure; PP, pulse pressure.

Currently, it is accepted that hypertension, diabetes mellitus, dyslipidaemias and smoking may increase aortic PWV, PPc and/or AIx, most likely through atherosclerotic processes. Before discussing the evidence in favour of indices of aortic stiffness independently predicting cardiovascular outcomes, it is important to first discuss why these indices may do so independent of traditional risk factors. Are indices of aortic stiffness simply a reflection of the extent of the underlying atherosclerotic process?

As indicated in the aforementioned discussion, aortic stiffness is an important determinant of an increased aortic systolic BP and PP, changes which themselves may predict cardiovascular outcomes beyond traditional risk factors (see section 1.3.1.2 for further explanation). Through increases in central aortic BP, aortic stiffness may account for significant cardiovascular morbidities such as left ventricular hypertrophy, and atherosclerotic vascular changes which ultimately translate into heart failure, myocardial infarcts, and strokes (Chirinos *et al*. 2011, Vlachopoulos *et al*. 2010, Mitchell *et al.* 2010). Measures of arterial stiffness may also represent the integrative effects of both the cumulative damage of traditional risk factors to the arterial wall over a long period of time and an individual's genetic background (Schnabel *et al.* 2008). Measures of aortic stiffness may therefore represent a surrogate end point that represents the temporal accrual of the adverse effects of traditional and genetic risk factors, whilst the traditional risk factors, which can fluctuate over time may not reflect their true accumulated impact on the arterial wall at the time of assessment (Vlachopoulos *et al.* 2010).

What is the evidence to show that aortic stiffness predicts cardiovascular outcomes and how is this information being used clinically? In this regard, there are a number of indices of aortic stiffening that may be employed and I will consider each in-turn.

1.3.1.1 Aortic pulse wave velocity and aortic stiffness

As biological structures increase in stiffness so does the speed that the pressure wave travels down that structure. Carotid-femoral (aortic) pulse wave velocity (PWV) is one such index of the speed of wave travel down the aorta and hence, is considered to be a close surrogate measure of aortic stiffness. Aortic PWV predicts cardiovascular outcomes independent of conventional risk factors (Vlachopoulos *et al.* 2010, Mitchell *et al.* 2010, Tsuchikura *et al.* 2010, McEniery *et al.* 2005, Weber *et al.* 2005, London *et al.* 2001, Meaume *et al.* 2001, van Popele *et al.* 2001). Due to its predictive value in estimating cardiovascular events and ease of measurement, aortic PWV is considered the "gold standard" measure of arterial stiffness (Laurent *et al.* 2006). Hence recent guidelines have included aortic PWV as a measure that may be employed for routine risk prediction and assessment of subclinical cardiovascular damage (Mancia *et al.* 2007). The European Society for Hypertension and European Society for Cardiology guideline recommend 12 m/sec as the upper limit of normal (Mancia *et al.* 2009), above which there is a 4% risk for a first major cardiovascular event within the following eight years (Mitchel *et al.* 2010). However, recently a lower PWV threshold of 10 m/sec has been suggested as the upper limit of normal (van Bortel *et al.* 2012). The lower velocity is based on a correction for errors of distance estimation between the common carotid artery and the commom femoral artery (van Bortel *et al.* 2012).

Despite the considerable evidence to support the view that aortic PWV predicts cardiovascular outcomes, there is nevertheless some controversy regarding changes in aortic PWV in atherosclerosis and in particular the changes that may occur in PAD. This controversy prompted me to develop a hypothesis that I tested as part of my thesis which, as demonstrated in chapter 2, resulted in the generation of a novel index which could conceivably be used as a risk predictor for CLI. The controversy and the hypothesis tested are discussed in section 1.4.0.

1.3.1.2 Central aortic pressures, augmentation index and aortic stiffness.

In comparison to the impact of static BP (diastolic and mean arterial BP), there is considerable evidence to indicate that dynamic BP (PP [systolic-diastolic BP]) and the effect of PP on systolic BP is a superior predictor for the development of both ischaemic heart disease and cardiac failure (Franklin *et al.* 1999). Moreover, in comparison to the impact of brachial systolic BP, there is evidence to show that central aortic dynamic pressures and systolic BP are more closely associated with cardiovascular outcomes and cardiovascular damage (Safar *et al.* 2002, Chirinos *et al.* 2005, Williams *et al.* 2006, Roman *et al.* 2007, Jankowsky *et al.* 2008, Pini *et al.* 2008, Wang *et al.* 2009, Wang *et al.* 2010, Norton *et al.* 2012). In comparison to other novel risk indices, central aortic PP predicts cardiovascular risk beyond indices such as aortic PWV, intima-media thickness (IMT) and left ventricular hypertrophy (Wang *et al.* 2009).

The question arises as to how it is possible that dynamic as opposed to static BP and central aortic as opposed to brachial artery dynamic BP are more closely associated with cardiovascular outcomes? To understand this issue I will describe the factors that determine dynamic pressures (pulse pressure) and hence systolic BP in the aorta and in the brachial artery.

1.3.1.2.1 Central aortic pulse pressure

Assuming a constant vascular resistance and hence static BP (diastolic BP and mean arterial pressure), dynamic aortic pressure (PP and hence systolic BP) is the result of convergence of the forward traveling pressure wave, originating from systolic ventricular contraction, and the reflected pressure wave arriving back from the periphery (Westerhof *et*

al. 2008). Also assuming a constant vascular resistance, the forward traveling pressure wave is determined by stroke volume and aortic impedance (Tartière-Kesri *et al.* 2012). Although there is evidence that stroke volume may contribute (Weber *et al.* 2005), age-related increases in the magnitude of the forward pressure wave is however, largely determined by increases in aortic impedance (Schutte 2011 *et al.*, Sugawara *et al.* 2010). In this regard, aortic stiffness, primarily in the proximal aorta (Schutte *et al.* 2011, Sugawara *et al.* 2010), is the main determinant of age-related increases in aortic impedance. Thus with a stiffer proximal aorta, the forward pressure wave is markedly increased and this contributes toward an enhanced aortic PP. Hence, the closer relationship between dynamic pressures (PP) as compared to static pressures and cardiovascular outcomes (see above) could reflect the impact of cardiovascular risk factors on aortic stiffness.

In young, healthy large vessels the reflected wave does not contribute to PP or systolic BP as this wave only returns during diastole, thus increasing diastolic BP, and boosting coronary flow. However, later in life, the reflected wave returns sufficiently early that it coincides with the forward pressure wave and thus enhances PP and systolic BP (Boutouyrie *et al.* 1992). Beyond static pressures, the contribution of the reflected wave to systolic BP is affected by several factors. These are as follows: The speed of reflected pressure wave travel, as indexed by PWV, (Segers *et al.* 2009), presumably by producing an earlier reflected wave, thus increasing the probability of the forward and reflected waves coinciding, which will increase the contribution of the reflected wave to aortic systolic BP. In addition the contribution of the reflected wave to aortic systolic BP will also be determined by the distance to the reflection sites (Segers *et al.* 2009), which if closer to the aorta will presumably result in earlier wave reflection, thus increasing the probability of the forward and reflected waves coinciding. Furthermore, the magnitude of wave reflection may further increase the contribution of wave reflection to aortic PP and systolic BP (Sugawara *et al.* 2010). Although wave reflection is clearly determined by many factors, including some not discussed here (e.g.

height, heart rate, distending pressures), a principle determinant of the contribution of wave reflection to aortic PP is through the impact of aortic stiffness modifying the speed of wave reflection. Hence, wave reflection, the other key determinant of central aortic PP (other than the forward wave pressure), is considered to be largely driven by aortic stiffness (Murgo *et al.* 1980). Thus, it is not surprising that because the two key determinants of central aortic PP, the forward and the reflected pressure waves, are generally believed to mirror changes in aortic stiffness, that aortic PP is considered a surrogate measure of aortic stiffness. However, with respect to the reflected wave, more direct indices of wave reflection have also been developed, measures which are similarly considered to be surrogate measures of aortic stiffness.

What are the currently employed indices of wave reflection and do these indices also predict cardiovascular outcomes beyond traditional risk factors?

1.3.1.2.2 Augmentation index

Augmentation pressure is the increase in aortic pressure that occurs as a result of the reflected wave meeting the forward traveling wave, and augmentation index (AIx) is the ratio of augmentation pressure to PP (O'Rourke *et al.* 1970). Hence AIx is a measure of wave reflection and its influence on the forward traveling waves. The use of AIx avoids problems of expressing aortic augmentation pressures as absolute values. In this regard, calibration of central aortic BP in many devices designed to assess aortic pressures and the component waves, has limitations. In this regard, the aortic pulse wave is generated from pulse wave analysis conducted at the radial pulse, whilst the calibration of the aortic pulse wave is from brachial artery BP measurements. This ignores any potential amplification of pressure waves from the brachial to the radial artery. Decreases in AIx have been show to correlate with decreases in central aortic BP in clinical trials (London *et al.* 1994, Kelly *et al*. 2001) and AIx

predicts cardiovascular events independent of brachial BP (Vlachopoulos *et al.* 2010, Mithchell *et al*. 2010, Wang *et al*. 2010, Wang *et al*. 2009, Pini *et al.* 2008, Roman *et al.* 2007, Williams *et al*. 2006, Weber *et al.* 2005, Boutouyrie *et al*. 2002, Safar *et al.* 2002, London *et al*. 2001). However, AIx may not correlate with coronary artery disease (Hope *et al.* 2007, Hayashi *et al.* 2002).

1.3.2 Carotid intima media thickness

An often advocated measure of target organ damage is the thickness of the carotid intima and media measured using high frequency B-mode ultrasound. The first description of the use of high resolution ultrasound to differentiate the layers of an arterial wall was in 1982 (James *et al*. 1982). The authors of this study (James *et al*. 1982), called these lines the I (Intima) and M (Media) lines, which together make the I-M complex. The thickness of the I-M complex has been shown to correspond with the thickness of the intima and media layers on histological examination (Pignoli *et al*. 1988). In humans the IMT complex consists of approximately 20% intima and 80% media (Adams *et al*. 1995). In the distal common carotid artery (CCA), which is straight and has no branches, thus encouraging laminar flow, the intimal layer is very thin (approximately 0.02 mm thick) (Salonen *et al*. 1993). The distal CCA is used for IMT measurement, as laminar flow discourages plaque development and plaque development in this area is thus thought to reflect a late finding suggestive of advanced pathology (Solberg *et al*. 1971). The combined thickness of the intima and media in the carotid artery (intima-media thickness [IMT]) reflects lipid infiltration into intimal macrophage cells and hypertrophy of medial smooth muscle cells (Spence *et al*. 2004). Thus IMT reflects changes in a critical part of the atherosclerotic process. This process occur as follows:

The interaction of risk factors with the arterial wall initiates the atherosclerotic process, with endothelial dysfunction being the starting point for atheroma formation (Li *et al*. 2011). Currently, the most important recognised contributors to endothelial dysfunction are haemodynamic disturbances (hypertension), hypercholesterolaemia, diabetes mellitus and inflammation (Zhang *et al.* 2012, Lanberg *et al.* 2012, Li *et al*. 2011). Other aetiologic contributors include cigarette toxins, homocysteine, and a wide spectrum of infectious agents (Kwiatkowska *et al*. 2011, Grunfeld *et al*. 2009, Hsue *et al*. 2009, Sacre *et al*. 2012, Hsue *et al*. 2006). Monocytes are attracted to the disturbed endothelium and migrate into the subendothelial space. Here they mature into macrophages and cause upregulation of pattern recognition receptors such as scavenger receptors and toll-like receptors in the endothelium (Kumar *et al*. 2007). Scavenger receptors mediate the internalization of oxidised low density lipoprotein cholesterol (LDL), resulting in foam cell formation. This represents the earliest stage in the formation of fatty streaks. The toll-like receptors transmit activating signals that result in the release of cytokines, proteases, and vasoactive molecules. These signals activate macrophages and attract additional monocytes. As foam cells accumulate in the subendothelial space, they distort the overlying endothelium and may eventually even rupture through the endothelial surface (Ross *et al*. 1995). Chronic endothelial injury eventually results in endothelial dysfunction and increased endothelial permeability. Consequently low density lipoprotein cholesterol (LDL) accumulates in the subendothelial space of the intima (Cybulsky *et al.* 1991) resulting in a thickened intima. LDL has a pro-inflammatory and proatherogenic effect thus exacerbating the its own accumulation and thickening the intima even further (Gounopoulos *et al.* 2007).

The ability of IMT to measure an important component of the atherosclerotic process is reflected in the capacity of this measurement to predict cardiovascular risk. Indeed, the

greater the degree of sub-clinical atherosclerosis, as evidenced by IMT, the greater the risk of future cardiovascular events (Greenland *et al*. 2000, Taylor *et al*. 2003). Though carotid IMT has a poor correlation with traditional risk factors (O'Leary *et al*. 1999), it has a relative risk ratio for cardiovascular events of 3.15 (confidence intervals=2.19-4.52) (Spence *et al*. 2004). In view of the evidence to support the use of IMT as a risk marker, in 2000 the American Heart Association approved IMT as a coronary heart disease risk assessment tool (Greenland *et al*. 2000). Although carotid IMT predicts risk in patients that already have cardiovascular disease (Simons *et al*. 1999) a current conundrum is that IMT progression does not correlate with an increased risk (Lorenz *et al*. 2012). Importantly, carotid IMT is strongly associated with lower extremity atherosclerosis and PAD (Bots *et al*. 1997, Allan *et al*. 1997). However, ABI and not IMT is the currently accepted preferred screening tool for detecting PAD. However, not all guidelines recommend ABI for routine risk prediction, whereas some guidelines recommend IMT measurements (Mancia *et al*. 2007). As will be discussed in subsequent sections (see section 1.4.0) I have hypothesised that IMT may be an important screening tool for use in patients infected with the human immunodeficiency virus.

1.3.3 Left ventricular function and the use of serum markers of dysfunction

There are currently a variety of measures of cardiac function that have been evaluated for risk prediction in a wide range of patient populations or community samples. These include measures of both systolic and diastolic chamber and regional myocardial function. More sensitive and better load- and heart rate-independent indices of chamber and regional myocardial function continue to emerge all the time. This topic largely goes beyond the scope of the present thesis. However, there are presently indices of cardiac function which have been better studied than others, and for which there is extensive data available. These indices were assessed in the present thesis for the reasons given in section 1.4.0 below. These include

the simple and reliable measures of systolic chamber and myocardial function including ejection fraction (EF) and midwall fractional shortening (FSmid) and the index of diastolic function, transmitral early-to-late (atrial) ratio (E/A). A brief review of the role of these measurements in risk prediction will therefore be provided. However, before doing so it is important to consider what the current standard measures of left ventricular systolic and diastolic function reflect.

The most commonly employed measure of LV systolic chamber function is ejection fraction (EF), which represents the ability of the LV to eject a given stroke volume (SV) in the context of filling volumes or end diastolic volume (EDV) (EF=SV/EDV). This index therefore accounts for the Frank-Starling effect (the ability of the heart to pump generated by increasing filling volumes). To understand this index better, in heart failure SV may be normal, not because the heart muscle is functionally intact, but because a compensatory increase in blood volume and hence filling volume maintains SV within normal resting values. This effect is designed to maintain a normal cardiac output and hence prevent cardiogenic shock from occurring. The only means of detecting an abnormal LV systolic chamber function is therefore to divide SV by EDV (=EF).

What is now increasingly recognised, is that LV concentric hypertrophy occurs in a heart with myocardial systolic dysfunction in order to maintain a normal wall stress. This occurs by reducing internal dimensions and hence decreasing radius. As wall stress or tension in the wall of a heart is inversely proportional to radius, wall stress is reduced as radius decreases. The consequence of a reduction in wall stress is that LV chamber systolic function (EF) is maintained within normal ranges and myocardial systolic dysfunction is missed. To detect myocardial systolic dysfunction the extent of concentric LV remodelling must be accounted for. This may be determined by evaluating the fractional shortening of the LV chamber at the midwall of the LV (FSmid). Importantly, this index is not a measure of LV function that is considered to be LV load or heart rate-independent. Although a number of

additional measures of LV systolic function, that may be independent of LV load, heart rate and remodelling, have been introduced over the years, FSmid has stood the test of time, may adequately reflect in LV systolic function independent of the LV remodelling process, and hence was considered to be sufficient for the purposes of the present thesis.

It is accepted that heart failure is frequently associated with a preserved EF (systolic LV chamber function) and that in these circumstances, in the absence of structural causes of heart failure or high output states, the heart failure is attributed to abnormalities of diastolic function of the LV. Although there are a variety of indices of diastolic function that may be measured, a more commonly measured index is the ratio of early-to-late (atrial) transmitral velocity (E/A). When abnormalities of relaxation occur in the LV, instead of most LV filling occurring during the early diastolic period (E), LV filling relies more on atrial contraction (A), the consequence being that the E/A ratio decreases. There are nevertheless confounders with the use of the E/A ratio, such as when end diastolic stiffness of the LV increases, the E/A ratio tends to return to normal (pseudonormalisation) or even increase (restrictive filling pattern) as LV filling relies more on early diastolic pressure gradients across the mitral valve. Hence, LV diastolic dysfunction may result in either decreases or increases in E/A. Although there are a number of additional measures of LV diastolic function that have been introduced over the past decade or so to account for variations in E/A in different forms of LV pathology, E/A remains one measure of LV function which may be employed to detect LV diastolic functional abnormalities of the heart. Importantly, there is now significant evidence to indicate that EF, FSmid and E/A predicts outcomes prior to the development of heart failure.

At a community level, asymptomatic mild left ventricular systolic dysfunction (EF ≤50%) may exist in 6% and moderate to severe systolic dysfunction (EF ≤40%) in 2% of individuals (Redfield *et al*. 2003). Measures of left ventricular dysfunction, including EF and E/A are independent predictors of fatal and non-fatal cardiovascular events in low risk (Fagard *et al*. 2001, Iivanainen *et al*. 1997) and high-risk patients (Mishra *et al*. 2011).

Furthermore, FSmid also predicts cardiovascular morbidity and mortality independent of left ventricular hypertrophy, blood pressure and age (DeSimone *et al*. 1996). Both FSmid and E/A, have also been demonstrated to predict heart failure in asymptomatic patients (Aurigemma *et al*. 2001). Moreover, therapeutic interventions have been shown to slow or even prevent the progression of asymptomatic left ventricular systolic dysfunction (reduced EF) (Doughty *et al*. 1997, The SOLVD Investigators 1992) and hence should be sought in "at risk" populations (Murtagh *et al*. 2012). As a consequence of the evidence to support a predictive role for measurements of subclinical cardiac dysfunction, the European Society of Cardiology guidelines (Dickstein *et al*. 2008) and the American Heart Association guidelines (Hunt *et al*. 2009) recommend their use in risk predicting.

Different risk prediction indices abound in the literature to predict which patients may suffer a perioperative adverse cardiac event. The Revised Cardiac Risk Index (Lee *et al.* 1999) is one of these indices and has been adopted into the American Heart Association and the European Society of Cardiologists cardiovascular assessment algorithms (Fleisher *et al*. 2007, Poldermans *et al*. 2009). This index includes six variables, two of which are the presence of ischemic heart disease and a history of congestive cardiac failure, however, it performs poorly in patients that are at intermediate cardiovascular risk (post-test probability =0.9) (Ridley 2003). B-type natriuretic peptides have been shown to be increased in patients undergoing major non-cardiac surgery who have adverse perioperative cardiac outcomes compared to patients in whom these peptides fall within normal reference ranges (Rodseth 2009, Rodseth *et al*. 2008). These natriuretic peptides have also been shown to improve risk stratification of intermediate risk patients into high or low risk groups in conjunction with the Revised Cardiac Risk Index (Biccard *et al*. 2011). Serum N-terminal pro-B-type brain natriuretic peptide (NT-proBNP), a natriuretic hormone released from the ventricles in response to increased filling pressures, has been shown to parallel the clinical severity of heart failure as assessed by the New York Heart Association functional class in broad populations

(Maisel *et al*. 2001). In addition, NT-proBNP concentrations correlate with a decreased LV EF (Troughton *et al*. 2000). Screening of asymptomatic patients for a decreased EF in a population where the prevalence of decreased EF is greater than 1% has been shown to be cost effective (Heidenreich *et al*. 2004). Conventionally an increased NT-proBNP concentration may lend weight to a clinical diagnosis of heart failure, or, may trigger a process to rule out heart failure when the diagnosis is unclear (Hunt *et al*. 2009, Wright *et al*. 2003).

1.4 **Role of novel approaches to predicting cardiovascular risk in peripheral arterial disease.**

As indicated in the introduction to section 1.3 above, in this thesis I explored novel approaches to the use of measures of large vessel structure (carotid intima-media thickness) and function (indexes of aortic stiffness) and left ventricular function as possible risk markers in advanced PAD. In the previous section I briefly described our current understanding of the use of these measures as risk predictors in the context of what is essential for our understanding of the hypotheses generated in this thesis. In this section I will describe what is understood regarding these novel risk factors in PAD and I will put forward hypotheses for exploring the novel use of these risk markers in advanced PAD.

1.4.1 Indices of arterial stiffness and peripheral arterial disease

As highlighted in section 1.3.1, aortic stiffening, as indexed by an increase in carotid-femoral PWV, predicts cardiovascular outcomes independent of conventional cardiovascular risk factors (O'Rourke *et al.* 2002). In section 1.3.1 I emphasized that there are two potential explanations for this relationship. First, aortic stiffness is a principle determinant of central

aortic BP and aortic BP predicts cardiovascular outcomes beyond brachial BP (Safar *et al*. 2002, Chirinos *et al*. 2005, Williams *et al*. 2006, Roman *et al*. 2007, Jankowsky *et al*. 2008, Pini *et al*. 2008, Wang *et al*. 2009, Wang *et al*. 2010). Second, indexes of aortic stiffness closely reflect the extent of atherosclerosis (van Bortel *et al*. 2012, Vlachopoulos *et al*. 2010, Tsuchikura *et al*. 2010). However, as indicated in Table 1.1, the aortic stiffness changes associated with PAD are contradictory and the key characteristics of studies demonstrating these changes or a lack thereof are summarised in Table 1.1. In the studies summarised in Table 1.1, the diagnosis of clinically significant PAD varies from clinical examination (Friberger 1912) to radiological evidence of arterial wall calcification (Ude 1933, Haynes *et al.* 1936), to ABI measurements combined with ischaemic symptoms (Yokoyama *et al*. 2003, Khandanpour *et al*. 2009). The age of the groups studied are similar except for 'young' patients included in the earliest study (Friberger 1912). The distance along which the pulse wave was measured differs significantly between studies; from subclavian artery to either radial (Friberger 1912) or femoral arteries (Ude 1933, Haynes *et al*. 1936), from carotid to femoral arteries (van Popele *et al*. 2001) or brachial to dorsalis pedis arteries (Yokoyama *et al.* 2003, Khandanpour *et al*. 2009). Importantly, the majority of studies summarised in Table 1.1 reported on findings obtained from small study samples, and hence may represent the effects of a selection bias or false positive findings. Nevertheless, as indicated in Table 1.1, two studies with larger sample sizes (n=643 and n=133) have provided evidence to show that PWV increases in PAD (Khandanpour *et al*. 2009, van Popele et al 2001). The presence of PAD was diagnosed either by reduced ankle-brachial indices (van Popele *et al*. 2001) or a reduced ABI together with claudication (Khandanpour *et al.* 2009). Moreover, in one prior study (van Popele *et al.* 2001), the extent of the increase in carotid-femoral PWV (mean difference=0.4 m/sec) relative to the absolute mean PWV (13-14 m/sec) in patients with PAD was clinically negligible, and in the other study (Khandanpour *et al*. 2009) brachial-knee and

brachial-ankle PWV, as opposed to carotid-femoral PWV were evaluated. These studies are heterogenous, and hence it is difficult to clearly determine how PWV is associated with PAD. In this regard, the impact of the inclusion of arm and leg together with aortic large artery measurements on PWV assessments is uncertain. Thus, despite their study sample size, these studies (van Popele *et al*. 2001, Khandanpour *et al*. 2009) have not necessarily reconciled the fact that there is controversy as to whether PAD is associated with increases in PWV.

In a recent systematic review, it was reported that PWV increases as the severity of atherosclerosis increases (Vlachopoulos *et al.* 2010), but this review failed to include literature prior to 1970. As some of the earliest studies have demonstrated that although PWV increases with age in normal arteries (Bramwell 1922, Simonson *et al*. 1955), this relationship is lost in atherosclerotic vessels (Bramwell 1922). In experimental studies, atheroma formation has been associated with either an increased or reduced aortic PWV (Rutherford *et al.* 1997, Farrar *et al*. 1991). Moreover, patients with marked hypercholesterolaemia may exhibit an increased rather than reduced large artery compliance, and thus a reduced PWV (Dart *et al*. 1991, Lehmann *et al*. 1992, Bots *et al.*1997, Allan *et al*. 1997). In addition, PWV decreases in SLE (Lee *et al.* 2006), a disease process often associated with extensive atheroma. Furthermore, although positive relationships have been described between the extent of atherosclerosis in the carotid arteries and large vessel stiffness or the presence of atherosclerotic disease and PWV (van Popele *et al.* 2001, Wada *et al*. 1994, Maarek *et al.* 1987), this relationship is inconsistent (Carlborg *et al*. 1944, Rawson *et al*. 1951, Simonson *et al.* 1955, Womersley *et al*. 1958, Eliakim *et al.* 1971, Nakashima *et al.* 1971, Riley *et al*. 1997, Megnien *et al*. 1998, Liao *et al.* 1999). Is there an alternative explanation as to why in PAD or in alternative examples of advanced atheromatous disease, aortic PWV may show variable changes?

Table 1.1. Summary of studies assessing pulse wave velocity in patients with peripheral arterial disease.

Author and year	Measurement technique	No with PAD	Diagnosis	Age (years)	Distance	Change in PWV (PAD vs control group)
Friberger 1912	Oscillometric pulse wave tracings	14	Clinically	'young'	Subclav-radial	Not significant
Ude 1933	Oscillometric pulse wave tracings	18	X-ray evidence of atherosclerosis	60-88	Subclav-femoral	Increased
Haynes *et al.* 1936	Oscillometric pulse wave tracings	9	X-ray evidence of atherosclerosis	44-78	Subclav-femoral	Increased
Simonson *et al.* 1955	Photoelectric digital volume pulse recording	24	Not described	45-54	Heart-dorsalis pedis	Decreased
Eliakim *et al.* 1971	Photoplethysmographic transducers	33	IC	>51	Femoral-dorsalis pedis	Decreased
van Popele *et al.* 2001	Oscillometric waveform analyzer	643	ABI <0.90	60-101	Carotid-femoral	Increased
Yokayama *et al.* 2003	Volume plethymography	9	ABI <0.90 & IC	67±7	Brachial-ankle	Increased
Khandanpour *et al.* 2009	Volume plethymography	133	ABI <0.90 & IC	45-85	Brachial-ankle	Increased
Tsuchikura *et al.* 2009	Oscillometric waveform analyzer	83	Previous vascular intervention	66±9	Heart-femoral	Increased

PAD, peripheral arterial disease; PWV, pulse wave velocity; IC, intermittent claudication; ABI=ankle-brachial index

Decreases in PWV in PAD or alternative examples of advanced atheromatous diseases may be attributed to a decline in distending pressures distal to arterial stenoses (Horrocks *et al.* 1979). This would result in an attenuation of arterial stiffness in these segments and hence may decrease PWV. Indeed, dampening of PWV distally in an atherosclerotic vessel as compared to the PWV in a proximal portion of the vessel is diagnostic of arterial stenoses or occlusion (Reneman 1981). If as a consequence of the presence of stenoses, PWV is reduced in advanced PAD, indexes of proximal aortic stiffness such as central aortic pulse pressure (PPc) may nevertheless remain increased. It is therefore plausible that a reduced aortic PWV in the context of increases in PPc (a PPc-PWV mismatch) may be a strong predictor of advanced atherosclerotic changes. In this thesis I therefore evaluated whether carotid-femoral PWV is indeed attenuated in advanced PAD (CLI), and if so, the extent to which this occurs, whether this attenuation translates into dissociation between carotid-femoral PWV and PPc, and whether a PPc-PWV mismatch index may be used to predict the presence of CLI independent of alternative indexes of large artery disease. In this regard, although in CLI the obstruction is often distal to where PWV is measured (carotid-femoral), CLI is typically multisegment disease (Norgren *et al*. 2007), and hence is often associated with stenoses proximal to the femoral artery. The hypothesis and aim of this study are outlined in section 1.5 below and the methodology and the data have been presented in chapter 2 of the present thesis.

1.4.2 Carotid intima media thickness and peripheral arterial disease

As discussed in a previous section (section 1.3.2) although carotid IMT is strongly associated with lower extremity arterial atherosclerosis and PAD (Bots *et al.* 1997, Allan *et al.* 1997), ABI and not IMT is the currently accepted screening tool for detecting PAD (Norgren *et al*. 2007). However, not all guidelines recommend ABI for routine risk prediction

(i.e. risk prediction regardless of whether IC exists), whereas some guidelines recommend IMT measurements (Mancia *et al*. 2007). As patients with atherosclerotic diseases, including PAD, have disease in other vascular beds (Mautner *et al*. 1992, Salonen *et al*. 1991, Newman *et al*. 1993) and the presence of disease in any vascular bed is associated with an increased incidence of overall cardiovascular events and mortality (Criqui *et al*. 1992, Nadelmann *et al*. 1990, Newman *et al*. 1993), when screening for cardiovascular risk in high risk patients, and not necessarily in patients with PAD, measures of target organ changes that reflect generalised atheromatous changes are required. In this regard, as discussed in section 1.3.2, carotid IMT is one such measure. However, there is considerable controversy regarding what changes in IMT mean when predicting cardiovascular risk in patients infected with HIV, patients who are at considerable risk for cardiovascular events. The following section describes this controversy and outlines additional studies that are required to advance this knowledge, studies which I have conducted in this thesis.

1.4.2.1 Cardiovascular disease in human immunodeficiency virus infections: What do changes in carotid IMT mean and how can studies in advanced peripheral arterial diseases expand our knowledge?

There is increasing evidence that infection with human immunodeficiency virus (HIV) is associated with occlusive arterial disease including myocardial infarction (Triant *et al*. 2007, Currier *et al*. 2003, Klein *et al*. 2002) and PAD (Periard *et al*. 2008, Ye *et al*. 2010, Qaqa *et al*. 2011, Giannarelli *et al*. 2011). In association to PAD, between 20 and 26.5% of HIV positive patients have a decreased ABI (Periard *et al*. 2008, Qaqa *et al*. 2011). Human immunodeficiency virus infected patients may develop PAD often in the absence of traditional risk factors and generally 20 years earlier than HIV negative patients (Periard *et al*.

2008, Giannarelli *et al.* 2011). A number of mechanisms may explain the relationship between HIV infection and PAD.

Partly through antiretroviral therapy (specifically protease inhibitors), patients with HIV infections have an increased prevalence of conventional cardiovascular risk factors (van Vonderen *et al*. 2009). The consequence may be endothelial dysfunction (Hsue *et al*. 2004, Klein *et al*. 2005), and an enhanced degree of subclinical atherosclerosis as indexed by increases in carotid IMT (Hsue *et al.* 2004, Micieli *et al*. 2007, Gutiérrez *et al.* 2008, Grunfeld *et al*. 2009, Hulten *et al*. 2009, VanVonderen *et al.* 2009). However, vascular pathology in HIV may also occur through HIV infection *per se* (Kwiatkowska *et al*. 2011), an effect potentially mediated through the virus infecting the arterial vasculature (Tabib *et al.* 1992, Bobryshev *et al*. 2000), and increasing the chances of vascular inflammatory changes occurring either directly (Hsue *et al*. 2009), or through co-infections with for example cytomegalovirus, herpes simplex virus or Chlamydia pneumoniae (Lemström *et al*. 1995, Libby *et al*. 1997, Hsue *et al.* 2006, Sacre *et al*. 2012) which may influence plaque stability. Thus, increases in carotid IMT in HIV infected patients may not only index the degree of atherosclerosis, but also alternative large artery phenotypes (Spence 2006). The measurement of carotid IMT may therefore be particularly useful at predicting cardiovascular risk beyond conventional risk factors in HIV infected patients. Presently however there is considerable uncertainty as to whether increases in carotid IMT occur in HIV independent of conventional risk factors.

A recent meta-analysis (Hulten *et al*. 2009) and a large study (Grunfeld *et al.* 2009) not included in this prior meta-analysis (Hulten *et al*. 2009) have suggested that the conventional risk factor-independent effects of HIV or antiretroviral therapy on common carotid IMT are modest and that to some extent these positive relationships reflect a publication bias (Hulten *et al*. 2009). The previous meta-analysis (Hulten *et al*. 2009) identified 12 studies but after completing a systematic review of all cross sectional or

observational studies of subclinical and clinical atherosclerosis among HIV positive patients compared to healthy cohorts, a further five studies can be included since its publication. A summary of all 17 studies is listed in Table 1.2. I performed a further meta-analysis on these 17 studies. Unadjusted IMT measurements were used in this review. The outcomes were pooled using a random effects model (Dersimonian *et al*. 1986) and heterogeneity assessed by the I^2 test (Higgins *et al*. 2002). The outcomes of this meta-analysis are illustrated in Figures 1.3 to 1.6.

All studies measured common carotid artery IMT using B-mode ultrasound. All studies except three (Chironi *et al*. 2003, Grunfeld *et al*. 2009, Mondy *et al*. 2008) showed a trend for an increased IMT with HIV infection. When one considers all the studies together, the mean difference was 0.01 for HIV positive participants having a thicker IMT compared to HIV negative participants. In sub-group analyses, only participants receiving antiretroviral therapy (ART) maintained a significant mean difference (Figure 1.5), whereas studies that did not differentiate between participants receiving ART's had a lower mean difference (Figure 1.4), and studies that included only ART-naive participants had a negative mean difference (Figure 1.4). In other words HIV positive participants had lower IMT's than HIV negative participants if ART naive. There is significant heterogeneity in all forest plots (Figures 1-4). This is not unusual in meta-analyses of observational studies (Hulten *et al*. 2009). This may be explained by the number of small studies which themselves may reflect a publication bias; the use of cross-sectional or cohort study designs, or differences between population groups themselves.

Even with cautious interpretation of the data, there seems to be limited evidence that HIV infection, or the treatment thereof, is associated with a thicker IMT. This review demonstrates that traditional risk factors for cardiovascular diseases overshadow the role of either HIV itself, or the use of ART, in determining premature large vessel lesions. The present meta-

analysis corroborates the findings of a previous meta-analysis that HIV or the use of ART does not increase carotid IMT, or the odds of developing carotid plaque (Hulten *et al*. 2009).

Table 1.2. Cross-sectional studies comparing IMT in HIV positive participants to a control group.

First author	Year of Publication	n (no HIV)	n (HIV)	IMT (no HIV)	IMT (HIV)	p-value	ART and ARTnaiive	CD4 count
Hsue	2004	63	148	0.74±0.17	0.91±0.33	p=0.0001	Both	NM
Seminari	2002	16	43	0.5±0.15	0.45±0.12	p=0.02	Naive (n=15)	534±228
					0.67±0.12	<0.003	ART (28)	570±258
Chironi	2003	36	36	0.55±0.06	0.53±0.09	p>0.5	ART	352±173
Papita	2011	36	63	0.51±0.08	0.60±0.15	p<0.01	Both	NM
Kwiatkowska	2011	27	72	0.51±0.30	0.65±0.23	<0.0001	Both	NM
Hsue	2006	37	93	0.68±0.41	0.95±0.38	<0.001	Both	NM
Grunfeld	2009	5 749	433	0.88±0.16	0.86±0.19	0.17	Both	NM
Johnsen	2006	86	97	0.61±0.6	0.62±0.6	0.07	Both	390±119
Lorenz	2008	1 168	292	0.72±0.16	0.74±0.16	0.0107	Both	NM
Currier	2005	44	88	0.70±0.5	0.71±0.5	0.34	Naiive (n=44)	481±148
					0.69±0.5	0.80	ART (n=44)	530±155
Bongiovanni	2008	54	186	0.58±0.5	0.64±0.5	>0.5	Naiive (n=53)	421±32
					0.65±0.5	>0.5	ART (n=133)	453±200

First author	Year of Publication	n (no HIV)	n (HIV)	IMT (no HIV)	IMT (HIV)	p-value	ART and ARTnaiive	CD4 count
Kaplan	2008 (women only)	477	1 191	0.72±0.5	0.72±0.05	p=0.65	Both	404±174
Kaplan	2008 (men only)	309	550	0.77±o.5	0.75±0.5	p=0.06	Both	381±146
Lebech	2007	14	25	0.66±0.1	0.67±0.2	p>0.5	ART	654±257
Lekakis	2008	25	71	0.55±0.20	0.64±0.2	p=0.01	ART	443±246
Mondy	2008	50	50	0.68±0.2	0.68±0.2	p=0.96	ART	547
van Wijk	2006	14	37	0.61±0.4	0.75±0.6	<0.05	ART	604±105
Yaldzizli	2006	40	47	0.56±0.11	0.74±0.22	p=0.001	Both	414±274

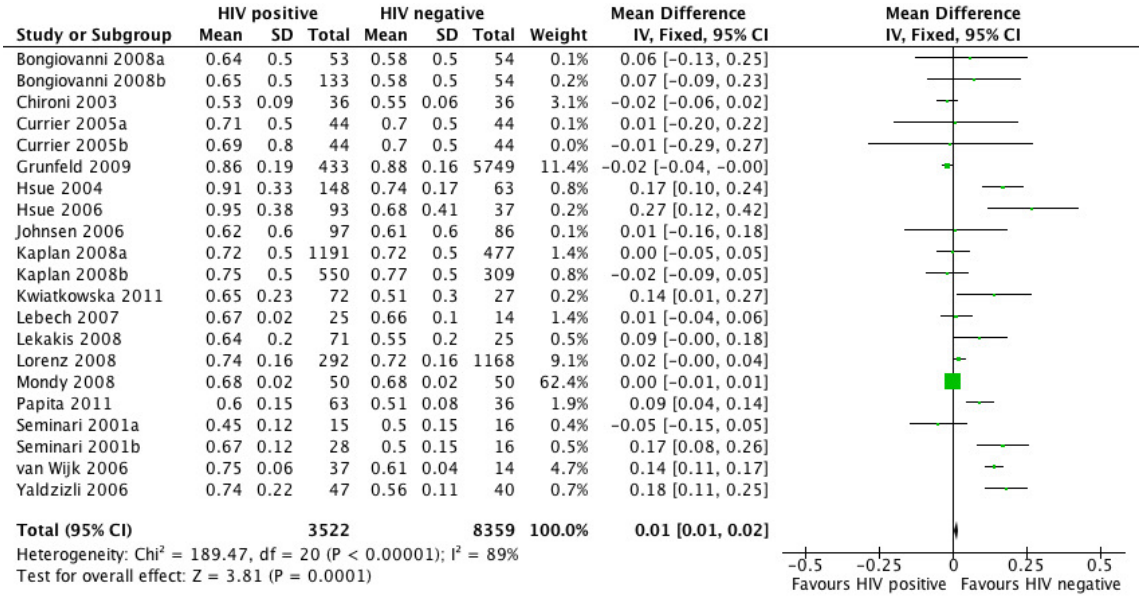

Figure 1.3. Forest plot of all 17 studies identified where carotid intima-media thickness was compared between HIV positive and negative participants.

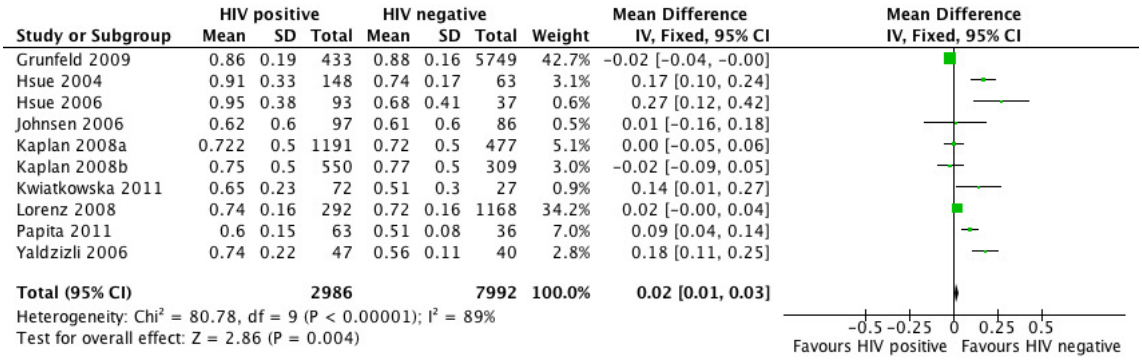

Figure 1.4. Forest plot of studies identified where carotid intima-media thickness was compared between HIV positive and negative participants that did not differentiate between participants receiving or not receiving antiretroviral therapy.

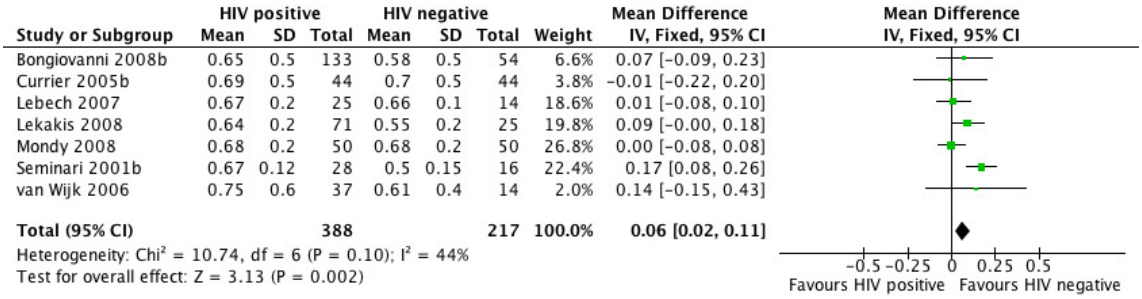

Figure 1.5. Forest plot of studies identified where carotid intima-media thickness was compared between HIV positive and negative participants which including participants receiving antiretroviral therapy only.

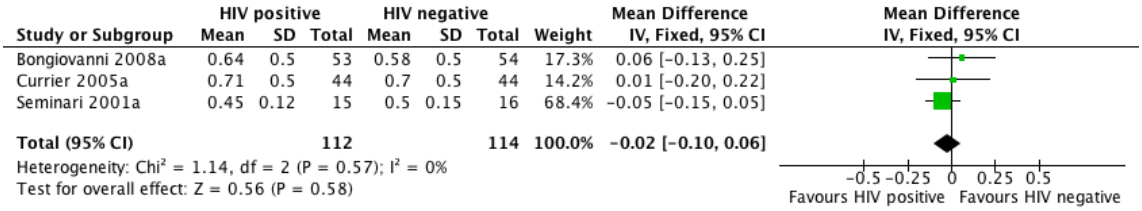

Figure 1.6. Forest plot of studies identified where carotid intima-media thickness was compared between HIV positive and negative participants which including participants who were antiretroviral therapy-naive only.

Irrespective of the strengths and weaknesses of prior studies assessing the extent to which HIV is associated with IMT independent of traditional cardiovascular risk factors, the results of the recent meta-analysis (Hulten *et al*. 2009), confirmed by an updated meta-analysis conducted by the myself, and the results of a large study (Grunfeld *et al*. 2009) suggest that increases in common carotid IMT previously reported on in HIV (Table 1.2) are more likely to be attributed to atherosclerosis mediated by conventional risk factors. Under these circumstances, this would argue in favour of cardiovascular risk being assessed mainly through conventional risk factors and would not provide a strong argument for assessing carotid IMT in addition to these risk factors. However, there are no studies that have explored the relationship between HIV and carotid IMT in patients with clinical manifestations of advanced occlusive arterial disease.

Recent studies have indicated that HIV infected patients in Africa presenting with occlusive arterial disease may have fewer risk factors for atherosclerosis, and angiographically or at surgery are more frequently noted to have a lower atherosclerotic burden (Mulaudzi *et al*. 2005, Becker *et al*. 2010). As HIV infection causes abnormalities of coagulation (Hsue *et al*. 2004, Grunfeld *et al*. 2009), which may contribute toward occlusive vascular disease (Robbs *et al*. 2009, Mulaudzi *et al*. 2005), HIV positive patients that present to vascular surgery services with ischaemic limbs in South Africa are not managed in the same manner as patients that present with typical atherosclerotic ischaemic limbs. HIV patients initially tend to have surgical thrombectomies, and rarely undergo primary endovascular procedures or open surgery as would patients with atherosclerotic PAD (Mulaudzi *et al*. 2005). In view of this approach it is important to establish whether a relationship between carotid IMT and PAD in HIV positive patients exists and whether this relationship is indeed independent of traditional cardiovascular risk factors. Consequently, in this thesis I explored the extent to which IMT is increased independent of conventional risk factors in African patients with chronic critical lower limb ischemia (CLI) who are HIV

infected as compared to those without evidence of HIV. The hypothesis and aim of this study are outlined in section 1.5 and the methodology and the data are presented in chapter 3.

1.4.3 The importance of left ventricular dysfunction in peripheral arterial disease

As reviewed in section 1.3.3, the presence of aymptomatic left ventricular dysfunction predicts the subsequent development of heart failure. Heart failure is associated with haemodynamic disturbances, which as discussed in section 1.1.6, contributes toward an increased perioperative mortality in PAD and toward a reduced patency of endovascular interventions. In this thesis I have raised the question as to whether asymptomatic left ventricular dysfunction is common in patients with PAD requiring surgery, and whether these changes are also associated with haemodynamic changes that could contribute toward a reduced perioperative survival and patency of endovascular interventions. In order to understand the reasons for posing this question I will review the evidence to show that the prevalence of heart failure is increased in PAD and that this is an important prognostic feature. Thereafter I will address the issue of why I have argued that asymptomatic left ventricular dysfunction may contribute toward worse outcomes in PAD.

As indicated in section 1.1.2, in patients with a previous lower limb amputation as a consequence of severe PAD, 92% of these patients had atherosclerotic plaques in their coronary vessels causing severe stenosis (75-99% of the lumen) or complete occlusion of these vessels (Mautner *et al*. 1992). Moreover, a prospective registry demonstrated that 11.6% of patients with IC will die from a myocardial infarction or stroke within two years of diagnosis (Stansby *et al*. 2011). In a cross-sectional community-based study of elderly participants, it was noted that 58% patients with IC had also suffered either a previous myocardial infarction or angina pectoris that required an invasive therapeutic intervention (Aronow *et al*. 1994). As it is well established that coronary artery disease is a significant

cause of heart failure in developed countries (Hunt *et al*. 2009), it should also be no surprise that the presence of PAD is associated with at least a two-fold increase in the prevalence of heart failure as determined from clinical signs and symptoms (Anand *et al*. 2007, Conrad *et al*. 2006, Bakken *et al*. 2009, Apelqvist *et al*. 2011, O'Brien-Irr *et al*. 2011). Is heart failure in PAD associated with a worse outcome?

As discussed previously, patients with heart failure and PAD have a higher two-year mortality rate (35.7%±4.5%%) than patients with PAD and no heart failure (17.7%±1.8%) (Meltzer *et al*. 2012). In addition, the success of interventions for PAD (endovascular or open vascular procedures) depend on several factors, amongst which is an adequate inflow of blood. This is determined by local vascular factors, such as a proximal stenosis or occlusion, as well as systemic factors such as cardiac output. Heart failure may result in a decreased cardiac output. In this regard, the primary patency of endovascular interventions is markedly reduced in patients with heart failure and this occurs in patients with a reduced EF (Meltzer *et al*. 2012). Hence the presence of heart failure plays a significant role in the outcome of patients requiring surgery for advanced PAD and the prognostic value of the presence of heart failure symptoms on postoperative outcomes is acknowledged in recent guidelines (Fleisher *et al*. 2007, Poldermans *et al*. 2009). However, the majority of patients with PAD do not have heart failure. Nevertheless, a significant number of patients with PAD may be asymptomatic and yet have left ventricular dysfunction. This does not preclude these patients from having early heart failure as an inability to walk without claudicantion will reduce the chances of eliciting symptoms of shortness of breath on exercise. The question therefore remains as to whether asymptomatic patients with left ventricular dysfunction may develop worse outcomes when requiring surgery for PAD, and if so what is the prevalence of asymptomatic left ventricular dysfunction in these patients?

Despite the evidence that favours a lack of benefit of evaluating left ventricular function prior to surgery (Wijeysundera *et al*. 2011), asymptomatic decreases in EF are

associated with a greater risk of long-term cardiovascular mortality after non-cardiac open vascular surgery (hazards ratio=4.6) including surgery for PAD (Flu *et al*. 2010). Moreover, the reduced patency of peripheral endovascular interventions associated with the presence of heart failure in PAD (Anand *et al*. 2007, Conrad *et al*. 2006, Bakken *et al*. 2009, Apelqvist *et al*. 2011, O'Brien-Irr *et al*. 2011) has largely been attributed to a reduced left ventricular EF (Meltzer *et al*. 2012). Indeed, in patients with heart failure, the patency of endovascular interventions after one year of follow-up in those with an EF <40% was 43.2%±3.5% compared to 56.6%±4.1% in patients with an EF ≥40% (Meltzer *et al*. 2012). It is therefore possible that a preoperative echocardiographic assessment may be useful to identify those that may benefit from therapy that may improve cardiac function, when planning vascular surgery in patients with CLI. Nevertheless, before considering this possibility it is important to establish the prevalence of asymptomatic left ventricular systolic dysfunction independent of clinical evidence of coronary artery disease and the haemodynamic or left ventricular structural features that characterize decreases in EF in unselected patients with PAD about to undergo surgery.

In regards to this is there evidence to support a hypothesis that haemodynamic changes may translate into altered outcomes in patients undergoing surgery for PAD?

1.4.3.1 What is the prevalence of asymptomatic left ventricular dysfunction in peripheral arterial disease and what are the characteristic haemodynamic features?

Prior studies conducted in select clinical samples have reported wide prevalence rates of a reduced EF, and it is uncertain to what extent this was associated with symptoms of heart failure or clinical evidence of coronary artery disease. Some key features of these studies are given in Table 1.3. A prevalence rate of 15-42% of patients with PAD requiring surgery with an EF <35-50% has previously been reported on (Rossi *et al*. 1998, Shrikhande *et al*. 2007,

Flu *et al*. 2010, van Kuijk *et al*. 2010, Iida *et al*. 2012, Table 1.3). However, in these studies 33-89% of patients had CAD (Rossi *et al*. 1998, Shrikhande *et al*. 2007,Flu *et al.* 2010, van Kuijk *et al*. 2010, Iida *et al*. 2012, Table 1.3), in three studies 20-67% of participants had a prior history of heart failure (van Kuijk *et al*. 2010, Shrikhande *et al*. 2007, Franco *et al*. 1989) and in one study 24% had a history of heart disease (Iida *et al*. 2012). In addition to prior studies (Rossi *et al*. 1998, Shrikhande *et al*. 2007,Flu *et al.* 2010, van Kuijk *et al*. 2010, Iida *et al*. 2012, Table 1.3) studies, one study reported a prevalence of an EF <55% as being 89% (Franco *et al*. 1989). The reasons for the discrepancy between this (Franco *et al.* 1989) and prior (Rossi *et al*. 1998, Shrikhande *et al*. 2007,Flu *et al*. 2010, van Kuijk *et al*. 2010, Iida *et al*. 2012, Table 1.3) studies is not obvious.

In none of the studies summarised in Table 1.3 were the haemodynamic changes associated with left ventricular dysfunction reported on. It is likely that if asymptomatic decreases in EF are to translate into a greater risk of long-term cardiovascular mortality following non-cardiac open vascular surgery including surgery for PAD (Flu *et al*. 2010), or a reduced patency of peripheral endovascular interventions associated with a reduced left ventricular EF (Meltzer *et al*. 2012), that haemodynamic disturbances such as a reduced cardiac output should accompany the reduced EF. In this regard, we cannot assume that this will be the case as haemodynamic changes are thought to distinguish symptomatic from asymptomatic patients with a reduced EF (Konstam *et al*. 1992), and in general the prevalence rate of severe dysfunction (Table 1.3), which is more likely to be associated with haemodynamic disturbances, appeared to be relatively low.

Table 1.3. Summary of main features of studies reporting on the prevalence of possible asymptomatic reductions in left ventricular ejection fraction in patients requiring surgery for peripheral arterial disease.

Studies	Patients	Sample size	Definition of reduced EF (EF<)	Prevalence	% Heart failure†	% CAD
Franco *et al.* 1989	PAD	85	<55%	89%	NM	NM
Rossi *et al.* 1998	PAD	114	<50%	42%	NM	NM
Shrikhande *et al.* 2007	PAD	270	<35%	20%	67%	89%
Flu *et al.* 2010	Vascular patients*	1005	<50%	19%	0%	53%
van Kuijk *et al.* 2010	PAD	1321	<50%	17-24%	NM	37-48%
Iida *et al.* 2012	CLI and isolated BK	406	<45%	15%	24%	52%

EF, ejection fraction; †symptoms or diagnosis or heart disease; CAD, coronary artery disease ; *including for PAD (35%) ; NM, not mentioned ; CLI, chronic critical limb ischaemia ; BK, below- knee;

Thus, in this thesis I assessed the prevalence of asymptomatic decreases in EF in unselected consecutive patients with PAD without clinical evidence of coronary artery disease requiring surgery for advanced PAD, and the haemodynamic changes associated with reductions in EF. Given the associations between circulating BNP concentrations and EF described in section 1.3.3 above, I also assessed whether circulating BNP measurements, which are advocated for risk prediction in patients with PAD requiring surgery (Goei *et al*. 2011), will identify patients with asymptomatic reductions in EF. The hypothesis and aims of this study are outlined in section 1.5 and the methodology and the data have been presented in chapter 5.

1.5 Summary of problem statements, hypotheses generated and aims of this thesis

In this thesis I have tested 3 hypotheses in patients with CLI. In this section I will summarise the problem identified which generated each hypothesis and consequently state the hypotheses tested and the aim of each study

1.5.1 Study 1: Arterial stiffness mismatch in critical limb ischaemia

As highlighted in section 1.1.5, given that up to 55% of patients with CLI are asymptomatic (no claudication) in the six months preceding their amputation (Dormandy *et al.* 1994), it is obvious that it is difficult to predict who will develop this stage of PAD. Although ABI measurements provide some degree of ability to identify patients with PAD who may come to amputation (Marston *et al*. 2006), ABI measurements are not recommended by all guidelines for routine risk prediction. In contrast aortic PWV measurements have been recommended for routine risk prediction by some guidelines (Mancia *et al*. 2007). However, as discussed in section 1.4.1 it is currently unclear whether increases in carotid-femoral PWV

measurements are associated with PAD. Indeed, PWV may be reduced in advanced PAD (see section 1.4.1) possibly because of the presence of stenoses proximal to the femoral artery. In contrast, although not examined, indexes of proximal aortic stiffness such as augmentation index (AIx) and wave reflection may nevertheless remain increased in advanced PAD. It is therefore plausible that a reduced aortic PWV in the context of increases in PPc (a PPc-PWV mismatch) may be a strong predictor of advanced atherosclerotic changes. I therefore **hypothesised** that because carotid-femoral PWV may be attenuated in advanced PAD (CLI) whilst PPc remains increased, that this translates into a dissociation between carotid-femoral PWV and PPc, and hence that a PPc-PWV mismatch index *may* be employed to predict the presence of CLI independent of alternative indexes of large artery disease. In chapter 2 of this thesis I therefore **aimed** to evaluate i) if carotid-femoral PWV is indeed attenuated in CLI and the extent to which this occurs, ii) whether this attenuation translates into a dissociation between carotid-femoral PWV and PPc, and if so whether iii) a PPc-PWV mismatch index may be used to predict the presence of CLI independent of alternative indexes of large artery disease.

1.5.2 Study 2: Carotid intima media thickness in patients with critical limb ischaemia and human immunodeficiency virus infection

Although there is no question that carotid intima media thickness to a large extent reflects atherosclerotic processes and is increased in patients with PAD (section 1.3.2), as discussed in section 1.4.2.1, the implications of increases in IMT in patients with HIV infections are uncertain. In section 1.4.2.1 I highlight the fact that increases in IMT could be attributed to the HIV infection *per se*, but that recent meta-analyses and large studies support the contention that increases in IMT in HIV infected patients are largely attributed to the high prevalence of co-existing traditional risk factors. Moreover, as also described in 1.4.2.1 there

is evidence, particularly in PAD, that occlusive arterial disease in young HIV infected patients is through arterial thromboses, rather than advanced atheroma formation. Thus, it is important to determine whether increases in IMT in HIV infected patients with occclusive arterial disease can be attributed to traditional cardiovascular risk factors. If IMT is increased, but this increase is not attributed to traditional risk factors, then IMT may prove to be very useful in identifying HIV infected patients at risk for advanced PAD beyond traditional risk factors. This finding would also suggest that occlusive arterial disease in HIV may not be viewed as primarily an enhanced state of thrombosis. In this thesis I therefore **hypothesised** that carotid IMT is increased independent of traditional risk factors in patients with CLI infected with HIV. In chapter 3 I therefore **aimed** to determine whether carotid IMT is increased independent of traditional risk factors in patients with CLI infected with HIV.

5.3 Study 3: Asymptomatic left ventricular dysfunction in patients with critical limb ischaemia: Prevalence and haemodynamic characteristics

In section 1.1.2 I have emphasised that PAD is often associated with coronary artery disease. In section 1.4.3 I have highlighted the fact that because of the relationship between coronary artery disease and PAD, that patients with PAD often have heart failure. Moreover, in the aforementioned section (1.4.3) I have indicated that patients with PAD and heart failure have a poor perioperative outcome and that this may be caused by a reduced EF. I have also pointed out the fact that a reduction in EF may nevertheless go undetected in patients with advanced PAD, largely because they are immobile and hence may not perceive the characteristic symptoms that accompany early heart failure. Consequently, as also highlighted in section 1.4.3 the question arises as to whether asymptomatic left ventricular dysfunction may also be associated with poor perioperative outcomes? In this regard, I have given two reasons to support this theory in this section of the chapter. However, I have indicated that the

value of seeking left ventricular systolic dysfunction in patients with end stage PAD will depend to a large extent on the prevalence of asymptomatic dysfunction without associated coronary artery disease as well as the associated haemodynamic disturbances. Without reductions in cardiac output it is unlikely that left ventricular dysfunction, as determined from EF, will translate into reduced outcomes in patients with PAD requiring surgery. As these questions have not been adequately addressed (see data reviewed under section 1.4.3.1) in this thesis I therefore **hypothesised** that asymptomatic left ventricular dysfunction without evidence of coronary artery disease is prevalent in patients with CLI and that reductions in EF are associated with haemodynamic disturbances, including a reduced cardiac output. In chapter 4 I therefore **aimed** to determine whether asymptomatic left ventricular dysfunction without evidence of coronary artery disease is prevalent in patients with CLI and whether reductions in EF are associated with haemodynamic disturbances, including a reduced cardiac output. In this chapter I also **aimed** to asssess whether measures of circulating BNP concentrations, which are routinely performed for risk prediction in patients with CLI, can predict asymptomatic left ventricular dysfunction.

CHAPTER 2

Mismatch Between Pulse Wave Velocity and Aortic Pulse Pressure: A Novel Marker of Advanced Peripheral Arterial Disease.

Abstract

Carotid-femoral pulse wave velocity (PWV) may be reduced in advanced peripheral arterial disease (PAD), however, aortic stiffness may be increased through atherosclerotic changes. I therefore aimed to determine whether in the context of increases in central aortic pulse pressure (PPc), decreases in carotid-femoral pulse wave velocity (PWV) predict the presence of advanced PAD. Applanation tonometry and vascular ultrasound were used to assess carotid-femoral PWV, PPc and carotid intima-media thickness (IMT) in 1030 randomly selected healthy adults (community sample) and 217 patients with chronic critical lower limb ischaemia (CLI). With adjustments for confounders, participants with CLI had an increased carotid IMT ($p<0.0001$) and PPc ($p<0.0001$), but a markedly reduced PWV (m/sec)(CLI=4.38±3.14, Community sample=6.78±2.47, $p<0.0001$). PWV was strongly correlated with PPc ($r=0.53$, $p<0.0001$) in the community sample, but not in CLI ($r=-0.04$). A stiffness mismatch index (PPc/PWV) showed significantly increased values in participants with CLI over the full adult age range assessed. With carotid IMT, PPc or aortic augmentation index in the same regression model, an increase in the stiffness mismatch index (PPc/PWV) was independently associated with CLI ($p<0.0001$) and a PPc/PWV value greater than the upper 95% confidence interval in the community sample predicted CLI (odds ratio=27.1, $p<0.0001$). In conclusion, in the context of an increased PPc, carotid-femoral PWV is markedly reduced in patients with CLI. These results suggest that a stiffness mismatch index (PPc/PWV) may be a new risk marker for advanced PAD.

2. Introduction

Aortic stiffening, as indexed by an increase in carotid-femoral pulse wave velocity (PWV), predicts cardiovascular outcomes independent of conventional cardiovascular risk factors (O'Rourke *et al.* 2002). There are two explanations for this relationship. First, aortic stiffness is a principle determinant of central aortic blood pressure (BP) and aortic BP predicts cardiovascular outcomes beyond brachial BP (Safar *et al.* 2002, Roman *et al.* 2007, Jankowsky *et al.* 2008, Chirinos *et al.* 2005, Pini *et al.* 2008, Wang *et al.* 2009, Wang *et al.* 2010, Williams *et al.* 2006). Second, indexes of aortic stiffness may closely reflect the extent of atherosclerosis (Lacroix *et al.* 2012, O'Rourke *et al.* 2002). However, some small studies suggest that in peripheral arterial disease (PAD), a reduced rather than increased PWV may occur (Eliakim *et al.* 1971, Simonson *et al.* 1955, Rawson 1951), a change that may be attributed to a decline in distending pressures distal to arterial stenoses (Horrocks *et al.* 1979). This would result in an attenuation of arterial stiffness in these segments.

Although small studies suggest that PWV is reduced in PAD (Simonson *et al.* 1955, Rawson 1951, Eliakim *et al.* 1971), there is nevertheless conflicting evidence in this regard, with large studies demonstrating increases in PWV in less advanced PAD (van Popele *et al.* 2001, Khandanpour *et al.* 2009). If, as a consequence of the presence of stenoses, PWV is reduced in advanced PAD, indexes of proximal aortic stiffness such as central aortic pulse pressure (PPc) may nevertheless remain increased. It is therefore plausible that a reduced aortic PWV in the context of increases in PPc (a PPc-PWV mismatch) may be a strong predictor of advanced atherosclerotic changes. In this study I therefore aimed to evaluate i) if carotid-femoral PWV is attenuated in advanced PAD (critical lower limb ischemia [CLI]) and the extent to which this occurs, ii) whether this attenuation translates into a dissociation between carotid-femoral PWV and PPc, and if so whether iii) a PPc-PWV mismatch index may be used to predict the presence of CLI independent of alternative indexes of large artery

disease. As 55% of patients with CLI may be asymptomatic 6 months before surgery (Dormandy *et al*. 1994), new simple and reproducible tools for predicting CLI are required.

2.1. Methods

2.1.1 Study groups.

This study study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval numbers: M11-08-29, M02-04-72, M07-04-69, M12-04-108). Participants gave informed, written consent. Two hundred and seventeen consecutive patients with CLI were recruited from the Division of Vascular Surgery at the Charlotte Maxeke Johannesburg Academic Hospital and the Chris Hani Baragwanath Hospital, Johannesburg, South Africa. The presence of CLI was identified as ischaemic pain at rest for more than two weeks, or the presence of ulcers or gangrene attributable to occlusive arterial disease (Rutherford *et al.* 1997). Data obtained in patients with CLI were compared with data obtained in 1030 of 1191 healthy participants from a community sample older than 16 years of age. The community sample was obtained from randomly recruited nuclear families of black African descent with at least two parents or two siblings and living in the South West Township (SOWETO) of Johannesburg, South Africa using the population census figures of 2001 (Woodiwiss *et al*. 2009, Redelinghuys *et al*. 2010, Norton *et al*. 2012). In this regard, the Chris Hani Baragwanath Hospital serves the SOWETO community and patients attending the Charlotte Maxeke Johannesburg Academic Hospital are generally from a similar socioeconomic class and ethnic group as those living in the SOWETO community. In 161 participants of the community sample aortic PWV could not be measured because of

the presence of marked obesity. Carotid IMT was measured in a substudy of 429 participants without cardiovascular disease.

2.1.2 Demographic and clinical data.

A questionnaire was administered to obtain demographic information and each participant's medical history, the use of medication and tobacco and alcohol use (Woodiwiss *et al*. 2009, Redelinghuys *et al*. 2010, Norton *et al*. 2012). Obesity was defined as a body mass index (BMI) $\geq$30 kg/m^2. Blood tests were performed including a fasting lipid profile and glycated haemoglobin (HbA1c). Patients with a fasting plasma glucose concentration $\geq$7mmol/l, or in whom glucose-lowering agents were prescribed, were considered to have diabetes mellitus (DM). Brachial blood pressure (BP) was measured according to guidelines and taken as the mean of five measurements. Participants with a BP $\geq$140/90 mm Hg or those receiving antihypertensive medication were considered to have hypertension.

2.1.3 Pulse wave analysis.

After participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded by applanation tonometry, each during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia) (Norton *et al*. 2012, Shiburi *et al*. 2006) (Figure 2.1). Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV were discarded. The pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. From a

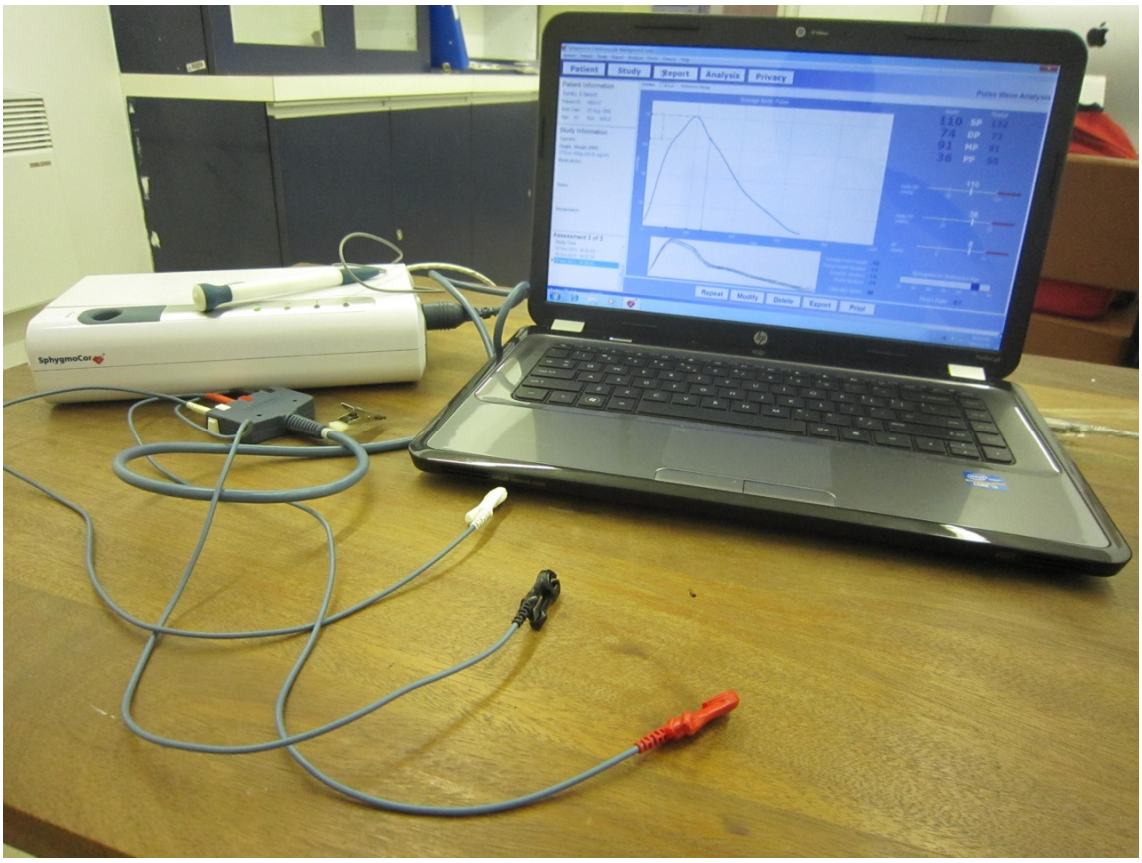

Figure 2.1. SphygmoCor device coupled to an applanation tonometer used to determine central (aortic) haemodynamics and aortic pulse wave velocity, with an image of radial artery and aortic pressure waves recorded from a participant demonstrated on the laptop (see Figure 1.2 for further details).

Validated inbuilt transfer function an aortic waveform was generated from which central systolic, diastolic and mean arterial BP were derived (Figure 1.2). The magnitude of the augmented pressure wave was determined as the difference between central systolic BP and the inflection point at the end of the first systolic shoulder. Central PP (PPc) was calculated as the difference between central systolic BP and central diastolic BP and mean arterial pressure (MAP) was calculated as [central diastolic BP + 1/3(central PP)]. Central augmentation index was determined as the augmented pressure wave/pulse pressure, expressed as a percentage.

Aortic PWV was measured from sequential waveform measurements at carotid and femoral sites as previously described (Shiburi *et al*. 2006) (Figure 1.1). Pulse wave transit time i.e. the time it takes the pulse wave to travel from the carotid to the femoral site, was determined as the difference between the times taken to generate the femoral and carotid pulse waveforms. To assess the differences in time of the generation of the femoral and carotid pulse waveforms, a single lead electrocardiogram was performed concurrently with pulse waveform sampling. The time delay in the pulse waves between the carotid and femoral sites was determined using the R wave as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance that the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch. Aortic PWV was calculated as distance (meters) divided by transit time (seconds).

2.1.4 Carotid intima-media thickness (IMT).

Common carotid artery intima media thickness was determined using a SonoSite (SonoCalc^{TM} IMT) version 3.4 through Doppler imaging (B-mode ultrasonography) with a high frequency linear probe (HFL38X/13-6 MHz) (Figure 2.2). Images of at least 1cm length of the far wall of the distal portion of the right common carotid artery from an optimal angle

of incidence (defined as the longitudinal angle of approach where both branches of the internal and external carotid artery or the bulb are visualized simultaneously)(Casella *et al.* 2008) at least 1 cm proximal to the flow divider or bulb were obtained. Images were obtained at least 1cm before the bifurcation or bulb as recommended (Bensen *et al*. 1999; Urbina *et al*. 2009) (Figure 2.3), as IMT increases closer to the bifurcation. The longest and clearest image of a flat segment of the common carotid artery, which was horizontally orientated on the ultrasound machine's screen was detected by rotating or keeling and towing (rocking the probe up and down lengthwise) the ultrasound probe. Care was taken to obtain a clear and non-tortuous image of the carotid artery that showed the near wall (top), the far wall (bottom) and the lumen.

Carotid IMT measurements were determined using semi-automated border-detection and quality control software over a length of 1 cm of intima-media. The patient's data and images were captured and saved on the ultrasound system (SonoSite MicroMaxx, USA), and transferred to a personal computer using SiteLink Image Manager for further analysis using the SonoCalc. program (Sonosite, Inc.).

The computerized edge detecting system (SonoSite MicroMaxx) uses algorithms to analyse ultrasound images (one at a time) and calculate IMT value(s). Using this edge detection software, the width of the intima-media, the mean thickness of the intima-media, and the maximum intima-media thickness were measured. This edge-detection software has become the accepted standard for carotid IMT measurement (Bots *et al.* 1997; Cobble *et al*. 2010), as the use of this software improves carotid IMT reproducibility and reduces observer bias compared with manual techniques. Care was taken to ensure that the measurement followed the contour of the intima-media layer precisely. A carotid IMT $\geq$0.8 mm was considered to be pathological (Veller *et al*. 1993).

Figure 2.2. The SonoSite (SonoCalc™ IMT) version 3.4 ultrasound MicroMaxx system with linear probe.

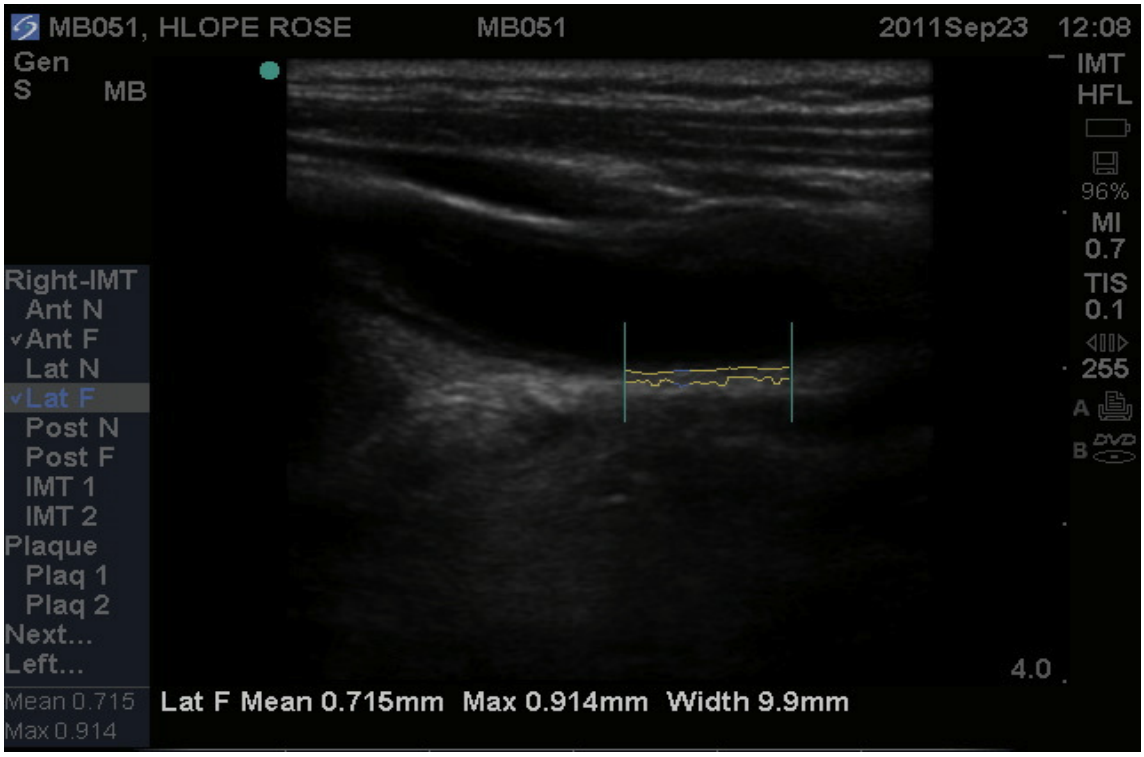

Figure 2.3. A B-mode ultrasound image of the measurement of the carotid IMT of the far wall as detected using the computerized edge detecting system that uses algorithms to analyze ultrasound (Doppler) images and calculate the IMT.

2.1.5 Data analysis.

Database management and statistical analysis was performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC). Multivariate regression analysis (linear when assessing continuous variables and logistic when assessing categorical variables) was performed with adjustments for age (when comparing mean values), sex, pulse rate, body height, mean arterial pressure, body weight, presence of hypertension, diabetes mellitus and smoking. As PWV was positively skewed (skewness=1.98, kurtosis= 7.30; Shapiro-Wilk's statistic=0.86, $p<0.0001$) PWV was log transformed. Log transformation of PWV resulted in an improved distribution (skewness=0.01, kurtosis= 1.13; Shapiro-Wilk's statistic=0.99). Correlations were compared using Z statistics. As more men than women had CLI, sensitivity analysis was performed in sex-specific groups. Although the majority of patients with CLI were of black African ancestry, some were of alternative ethnic groups. To ensure that ethnic differences did not confound the results, sensitivity analysis was also conducted only in participants whom were of black African ancestry. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood as implemented by the mixed procedure as defined in the SAS package.

2.2 Results

2.2.1 Participant characteristics.

Table 2.1 shows the demographic and clinical characteristics of the study samples. 62.7% of participants with CLI, and all the control participants were of black African descent. The patients with CLI were on average older, and a greater proportion were male. A greater proportion of participants with CLI had hypertension and DM and more participants with CLI

were receiving antihypertensive medication, glucose lowering therapy and lipid lowering agents. A greater proportion of participants with CLI regularly smoked. The characteristics of community participants in whom carotid IMT was measured were similar to the characteristics in those in whom this measurement was not performed (Table 2.2).

2.2.2 Comparisons of PWV.

With or without adjustments for confounders, PWV was markedly lower in participants with CLI than in the community sample from an age when PWV began to increase in the community sample (50-60 years) (Figure 2.6). With age included in the multivariate model, in all participants aortic PWV was markedly lower in participants with CLI and these differences were noted in both women and men and in participants of black African descent (Table 2.3). In addition, with the same adjustments, log PWV (m/sec) was markedly lower in patients with CLI than in the community sample (CLI=0.63±0.20, Community sample=0.80±0.16, $p<0.0001$) and these differences were noted in both women (CLI=0.56±0.30, Community sample=0.78±0.13, $p<0.0001$) and men (CLI=0.67±0.24, Community sample=0.81±0.20, $p<0.0001$) and in participants of black African descent (CLI=0.63±0.18, Community sample=0.79±0.14, $p<0.0001$).

Table 2.1. Characteristics of participants.

Characteristic	Community sample	Critical lower limb ischemia
Sample size	1030	217
% Black African	100	62.7
% Males	36	71*
Age (years)	43±18	62±12*
Height (metres)	161±9	170±10*
Body mass index (kg/m^2)	28.5±7.3	26.3±5.9*
% Obese	39	18*
% Hypertensive	42	62*
% receiving antihypertensives	23	62*
% with diabetes mellitus	8	45*
Current smoker (%)	16	51*
Previous smoker (%)	24	68*
Regular alcohol (%)	22	57*
Blood pressure (SBP/DBP)(mm Hg)	129±22/84±12	137±21*/80±12*
Pulse rate (beats/min)	65±12	84±17*
Glycated hemoglobin (%)	6.07±1.31	7.53±2.48*
Total/HDL cholesterol	3.49±1.24	4.04±1.69*

* $p<0.0001$ versus community sample. Data are shown as mean±SD or percentages. SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein.

Table 2.2. Characteristics of participants with and without carotid intima-media thickness (IMT) measurements in the community sample.

Characteristic	With IMT measurements	Without IMT measurements
Sample size	429	601
% Males	36.6	36.3
Age (years)	43.8±18.6	42.2±18.2
Height (metres)	161±8	161±9
Body mass index (kg/m^2)	28.9±7.7	28.3±7.0
% Obese	38.9	38.3
% Hypertensive	43.4	40.1
% receiving antihypertensives	24.9	22.0
% with diabetes mellitus	8.2	8.2
Current smoker (%)	16.6	15.8
Previous smoker (%)	24.0	23.6
Regular alcohol (%)	23.8	20.8
Blood pressure (SBP/DBP)(mm Hg)	128±22/83±12	129±23/84±12
Pulse rate (beats/min)	65±12	65±11
Glycated hemoglobin (%)	6.02±1.30	6.11±1.32
Total/HDL cholesterol	3.49±1.20	3.50±1.26

Data are shown as mean±SD or percentages. SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein.

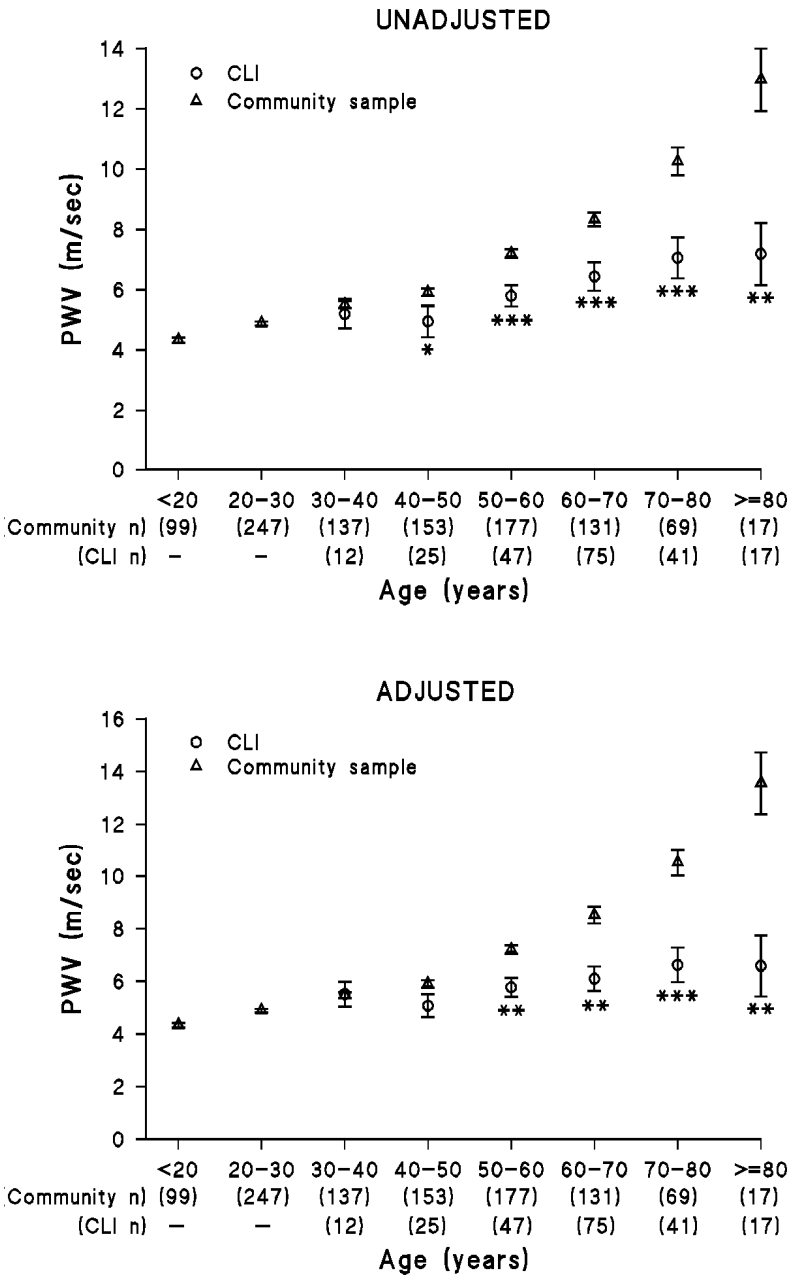

Figure 2.4. Unadjusted (upper panel) and multivariate adjusted (lower panel) age-related increases in carotid-femoral pulse wave velocity (PWV)(left panels) in adults from a randomly selected community sample (n=1030) and in participants with chronic critical lower limb ischemia (CLI)(n=217). Data are shown as mean±SEM. *p<0.05, ** p<0.005, *** p<0.0001 for CLI vs community sample. Adjustments are as given in Table 2.3.

Table 2.3. Comparison of multivariate adjusted measures of large artery structure and function between participants with chronic critical lower limb ischemia and healthy participants from a community sample.

Large artery structure and function	Community sample	CLI	p-value*
	All participants		
Carotid-femoral pulse wave velocity (m/sec)	6.78±2.47 (n=1030)	4.38±3.14 (n=217)	<0.0001
Aortic augmentation index (%)	26.3±10.0 (n=1030)	29.5±12.7 (n=217)	<0.005
Aortic pulse pressure (mm Hg)	35.2±11.8 (n=1030)	43.1±15.0 (n=217)	<0.0001
Carotid intima-media thickness (mm)	0.66±0.16 (n=429)	0.74±0.18 (n=217)	<0.0001
	Women		
Carotid-femoral pulse wave velocity (m/sec)	6.54±1.88 (n=655)	3.31±4.29 (n=63)	<0.0001
Aortic augmentation index (%)	28.4±9.0 (n=655)	30.2±20.5 (n=63)	=0.23
Aortic pulse pressure (mm Hg)	35.4±10.5 (n=655)	40.4±23.9 (n=63)	<0.005
Carotid intima-media thickness (mm)	0.65±0.11 (n=272)	0.72±0.26 (n=63)	<0.001

Adjustments are for age, sex (in all participants), pulse rate, body height, mean arterial pressure, body weight, presence of hypertension, diabetes mellitus, and smoking. *Probability values were further adjusted for non-independence of family members. Data are shown as mean±SD.

Table 2.3. continued. Comparison of multivariate adjusted measures of large artery structure and function between participants with chronic critical lower limb ischemia (CLI) and healthy participants from a community sample.

Large artery structure and function	Community sample	CLI	p-value*
	Men		
Carotid-femoral pulse wave velocity (m/sec)	7.13±3.22 (n=375)	5.04±3.85 (n=154)	<0.0001
Aortic augmentation index (%)	23.5±11.2 (n=375)	26.9±13.5 (n=154)	<0.05
Aortic pulse pressure (mm Hg)	34.1±13.6 (n=375)	45.8±16.3 (n=154)	<0.0001
Carotid intima-media thickness (mm)	0.67±0.20 (n=157)	0.76±0.20 (n=154)	<0.005
	Black African		
Carotid-femoral pulse wave velocity (m/sec)	6.66±2.16 (n=1030)	4.25±2.68 (n=136)	<0.0001
Aortic augmentation index (%)	26.5±9.3 (n=1030)	30.1±11.6 (n=136)	<0.001
Aortic pulse pressure (mm Hg)	35.2±11.3 (n=1030)	42.8±14.0 (n=136)	<0.0001
Carotid intima-media thickness (mm)	0.65±0.14 (n=429)	0.73±0.17 (n=136)	<0.0001

Adjustments are for age, sex (in all participants), pulse rate, body height, mean arterial pressure, body weight, presence of hypertension, diabetes mellitus, and smoking. *Probability values were further adjusted for non-independence of family members. Data are shown as mean±SD.

2.2.3 Comparisons of PPc, AIx and IMT.

In contrast to the lower PWV values noted in participants with CLI as compared to the community sample (Figure 2.4), as compared to the community sample, participants with CLI showed increased multivariate-adjusted PPc values over the age range 40-70 years (Figure 2.5). Moreover, with age included in the multivariate models, adjusted PPc, AIx and carotid IMT were higher in participants with CLI than in the community sample and these differences were noted in men and women and participants of black African descent for PPc and IMT and in men and participants of black Africn descent for AIx (Table 2.3).

2.2.4 Relationships between PWV and AIx, PPc or carotid IMT.

In the community sample strong bivariate correlations were noted between PWV and carotid IMT ($r=0.50$, CI=0.42 to 0.56, $p<0.0001$), AIx ($r=0.33$, CI=028 to 0.39, $p<0.0001$) or PPc (Figure 2.8), whilst these correlations were absent or markedly attenuated in participants with CLI (PWV vs IMT; $r=0.06$, CI=-0.07 to 0.19 $p=0.37$: PWV vs AIx; $r=0.13$, CI=-0.01 to 0.26, $p=0.06$)(PWV vs PPc , Figure 2.6). In contrast to the lack of relationship between PWV and PPc in participants with CLI, AIx was as well correlated with PPc in patients with CLI as it was in the community sample (Figure 2.8). Hence, the PWV-PPc, PWV-AIx, and PWV-IMT relationships in the community sample were considerably greater than that in participants with CLI ($p<0.05$ for PWV-AIx and $p<0.0001$ for PWV-PPc and PWV-IMT when comparing r values using the Z test), whilst the AIx-PPc relationship in participants with CLI was the same as that in the community sample ($p=0.27$ for comparison of r values using the Z test).

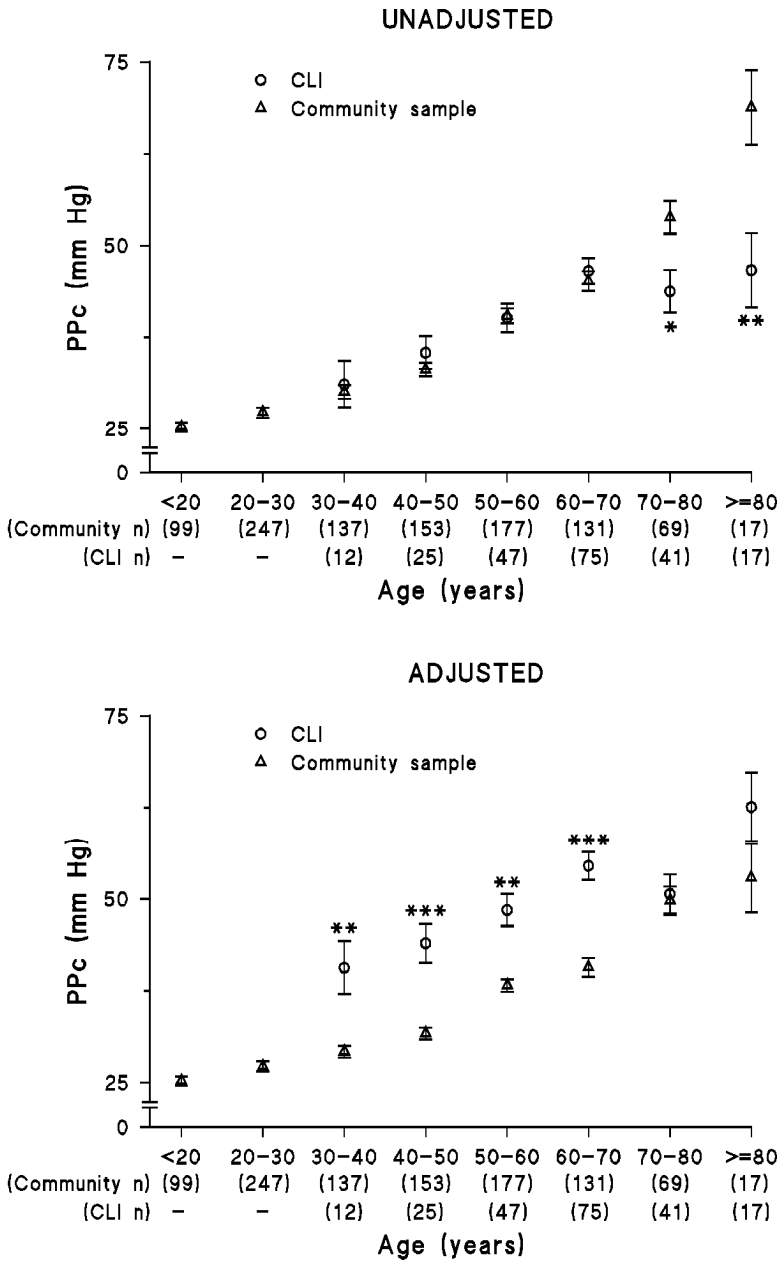

Figure 2.5. Unadjusted (upper panel) and multivariate adjusted (lower panel) age-related increases in central aortic pulse pressure (PPc)(right panels) in adults from a randomly selected community sample (n=1030) and in participants with chronic critical lower limb ischemia (CLI)(n=217). Data are shown as mean±SEM. *p<0.05, ** p<0.005, *** p<0.0001 for CLI vs community sample. Adjustments are as given in Table 2.3.

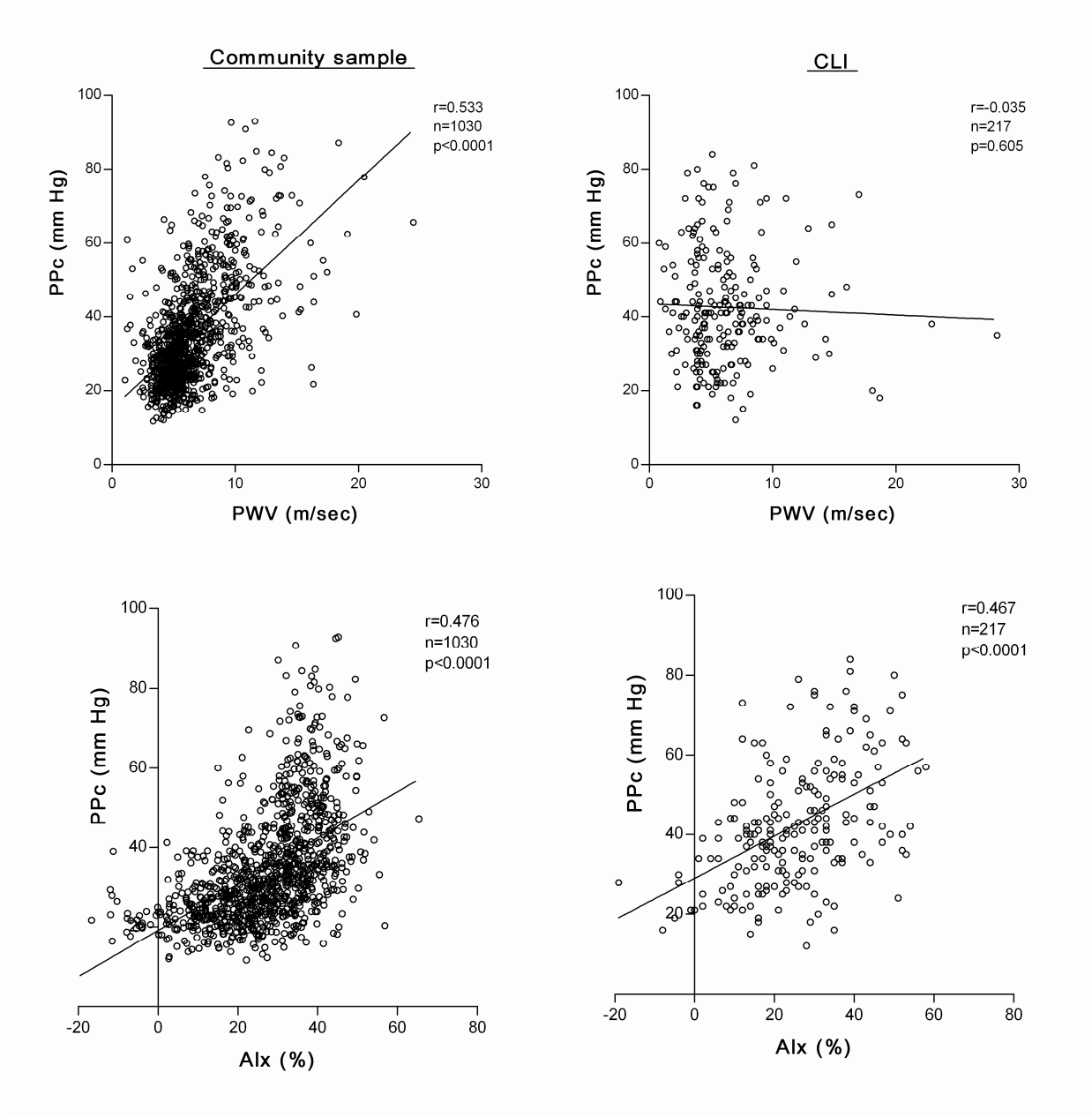

Figure 2.6. Relationships between aortic pulse wave velocity (PWV) or aortic augmentation index (AIx) and central aortic pulse pressure (PPc) in adults from a randomly selected community sample and in participants with chronic critical lower limb ischemia (CLI). Note the absence of a PWV-PPc, but the presence of a AIx-PPc relationship in CLI.

2.2.5 Associations of a stiffness mismatch index (PPc/PWV) with CLI.

An index quantifying the exent of the mismatch between PPc and PWV was generated as PPc/ PWV. This index was markedly increased in patients with CLI as compared to the community sample across all age-groups in unadjusted or multivariate-adjusted models and when assessed in women, men or black Africans only (Figure 2.7). With PPc/PWV together with either PPc, AIx or IMT included in the same multivariate regression models, an increased PPc/PWV emerged as being independently associated with CLI (Table 2.4).

The risk of having CLI was markedly increased in participants with a stiffness mismatch index (PPc /PWV) ≥9.66 (95% CI of 393 healthy participants of the community sample)(odds ratio=27.1, confidence intervals=10.6 to 69.0, p<0.0001) even with IMT (odds ratio=26.3, confidence intervals=10.1 to 68.7, p<0.0001), AIx (odds ratio=27.0, confidence intervals=10.6 to 69.0, p<0.0001) or PPc *per se* (odds ratio=19.5, confidence intervals=7.1 to 53.4, p<0.0001) included in the regression models and this effect was considerably greater than that for participants with an IMT ≥0.8 mm (odds ratio=4.08, confidence intervals=2.15 to 7.76, p<0.0001), a PPc≥40.1 (95% CI of 393 healthy participants of the community sample) (odds ratio=5.71, confidence intervals=3.02 to 10.80, p<0.0001), or an AIx≥41.4 (95% CI of 393 healthy participants of the community sample) (odds ratio=2.99, confidence intervals=1.45 to 6.17, p<0.0005).

As compared to either the age- and sex-matched controls, PPc/PWV showed a similar performance (area under the receiver operating curve) for CLI detection as compared to IMT or AIx, whilst PPc alone failed to show significant performance for CLI detection (Table 2.5.). However, PPc/PWV showed an increased sensitivity and specificity for CLI detection as compared to AIx, and an increased specificity for CLI detection as compared to IMT.

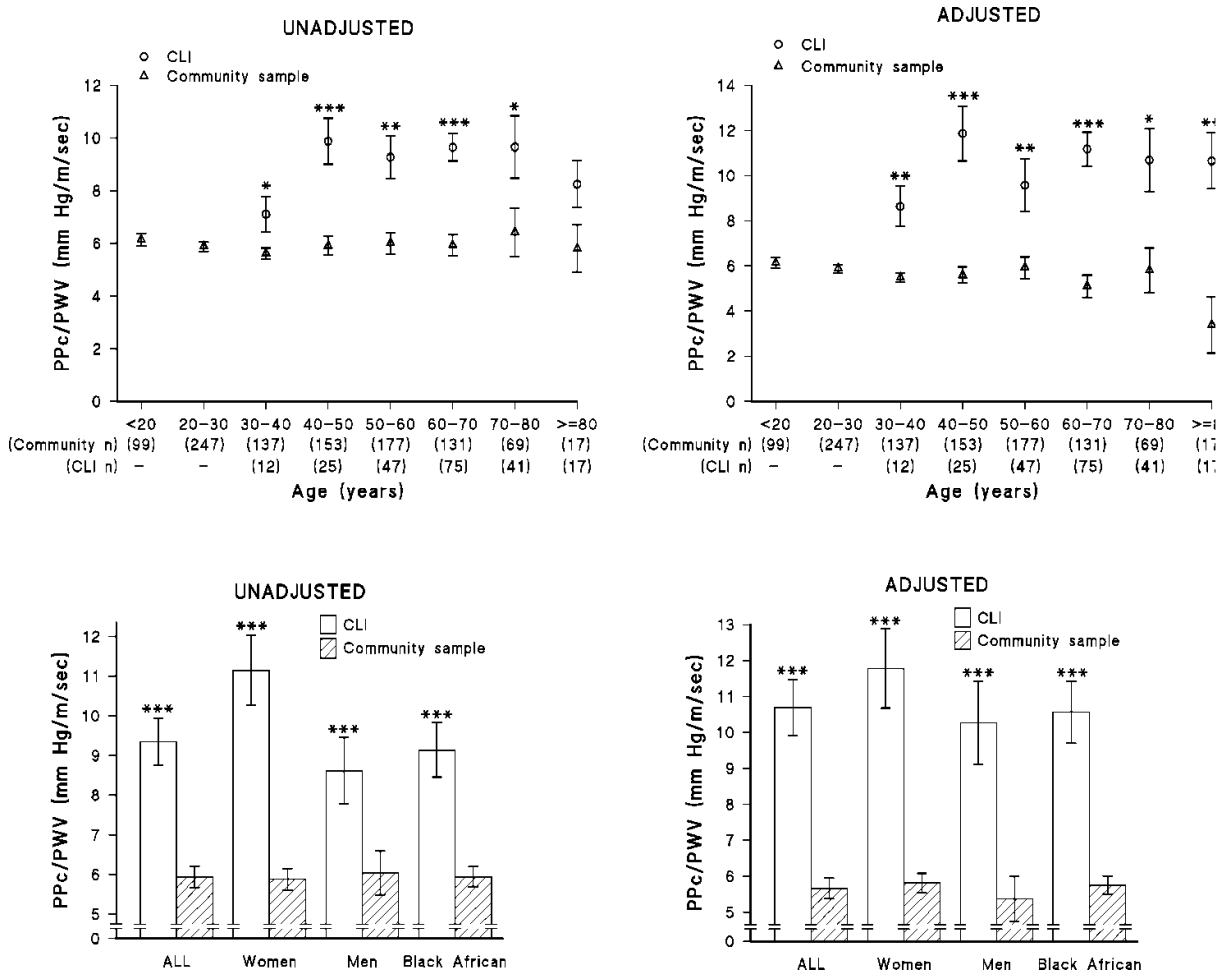

Figure 2.7. Unadjusted (upper panels) and multivariate adjusted (lower panels) differences in a stiffness mismatch index (central aortic pulse pressure [PPc]/ carotid-femoral pulse wave velocity [PWV]) in adults from a randomly selected community sample (n=1030) and in participants with chronic critical lower limb ischemia (CLI) (n=217). Data are shown as mean±SEM for the upper panels and mean and confidence intervals for the lower panels. *p<0.05, ** p<0.005, *** p<0.0001 for CLI vs community sample. Adjustments are as given in Table 2.

Table 2.4. Independent relationships between factors associated with chronic critical limb ischemia (CLI) with a stiffness mismatch index (central aortic pulse pressure [PPc]/carotid-femoral pulse wave velocity [PWV]), PPc, carotid intima-media thickness (IMT), or central aortic augmentation index (AIx) in the same regression models (n=217 with CLI versus 429 from a community sample).

CLI versus	β-coeff.* ±SEM	p- value†	β-coeff.* ±SEM	p- value†	β-coeff.* ±SEM	p- value†
PPc/PWV	0.17±0.03	<0.001	0.19±0.03	<0.0001	0.19±0.03	<0.0001
PPc	0.10±0.04	<0.05	-	-	-	-
Carotid IMT	-	-	0.16±0.04	<0.01	-	-
AIx	-	-	-	-	0.06±0.04	=0.48
Age	0.35±0.04	<0.005	0.31±0.04	=0.001	0.37±0.04	<0.0005
Regular smoking	0.20±0.03	<0.0001	0.18±0.03	<0.0001	0.19±0.03	<0.0001
Regular alcohol	0.12±0.03	=0.07	0.13±0.03	=0.05	0.12±0.03	=0.08
Diabetes mellitus	0.17±0.03	<0.0001	0.18±0.03	<0.0001	0.18±0.03	<0.0001
Pulse rate	0.40±0.03	<0.0001	0.37±0.03	<0.0001	0.39±0.03	<0.0001
Mean arterial pressure	-0.13±0.04	=0.005	-0.09±0.03	<0.05	-0.11±0.04	<0.05
Body height	0.32±0.04	<0.0001	0.32±0.04	<0.0001	0.32±0.04	<0.0001

* Standardized β-coefficient. Also included in the regression models were sex, body weight, and the presence of hypertension. None of these were significantly asociated with CLI. †Probability values were further adjusted for non-independence of family members.

Table 2.5. Sensitivity, specificity and performance (area under the receiver operating curve AUC) of a novel index of arterial function (PPc/PWV) versus alternative indices of arterial structure and function to predict the presence of critical lower limb ischaemia (CLI). Sample sizes: Age- and sex-matched controls from a randomly selected community sample=194 for arterial function and 88 for IMT; CLI=136.

CLL vs	Threshold	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	AUC (±SEM)
PPc/PWV	≥9.66	28.7 (21.3-37.1)	96.9 (93.4-98.9)	0.64±0.03**
Carotid IMT	≥0.8 mm	44.9† (36.3-53.6)	81.8†† (72.2-89.2)	0.68±0.04*
PPc	≥40 mm Hg	50.7†† (42.0-59.4)	47.9†† (40.7-55.2)	0.53±0.03†
AIx	≥41%	11.2†† (6.4-17.8)	76.3†† (69.7-82.1)	0.68±0.03**

CI, confidence interval; PPc, central aortic pulse pressure; PWV, aortic pulse wave velocity; IMT, carotid intima-media thickness; AIx, central aortic augmentation index. *p<0.005, **p<0.0001 for significance of AUC values. †p<0.05 ††p<0.005 vs PPc/PWV.

2.3 Discussion

The main findings of the present study are as follows: i) Unadjusted and multivariate adjusted carotid-femoral PWV was markedly attenuated in advanced PAD (critical lower limb ischemia [CLI]); ii) this attenuation translated into a dissociation between carotid-femoral PWV and PPc in that the strong correlations that were noted in the community sample were abolished in participants with CLI; and iii) an index, PPc/PWV (stiffness mismatch index), predicted the presence of CLI independent of and stronger than alternative indexes of large artery disease, including IMT, AIx and PPc *per se*.

Although an increased carotid-femoral PWV has been well described as preceding or accompanying cardiovascular diseases such as stroke and coronary artery disease (O'Rourke *et al.* 2002), evidence for such relationships in PAD are less well documented. This study provides data from a large study sample, using modern measurement techniques to support earlier small studies limited by a potential selection bias and using outdated technology (Simonson *et al.*1955, Rawson 1951, Eliakim *et al.* 1971), that suggest that PAD, as evidenced by CLI, is associated with a reduced PWV. These data are in contrast to some studies reporting on an increased PWV in patients with PAD (van Popele *et al.* 2001, Khandanpour *et al.* 2009). Nevertheless, in these studies (van Popele et al 2001, Khandanpour *et al.* 2009), the presence of PAD was identified from ankle-brachial indexes and in one study (van Popele *et al.* 2001) the presence of claudication or clinical evidence of arterial insufficiency was not required for the diagnosis. In contrast, this study was conducted in patients with CLI, who are likely to have had more severe atherosclerosis than those patients previously studied (van Popele *et al.* 2001, Khandanpour *et al.* 2009). Moreover, in a prior study (van Popele *et al.* 2001), the extent of the increase in carotid-femoral PWV (mean difference =0.4 m/sec) relative to the absolute mean PWV (13-14 m/sec) in patients with PAD

was clinically negligible, and in another study (Khandanpour *et al*. 2009) brachial-knee and brachial-ankle PWV, as opposed to carotid-femoral PWV were evaluated. In this regard, the impact of the inclusion of arm and leg together with aortic large artery measurements on PWV assessments is uncertain.

Despite decreases in PWV and a lack of relationship between PWV and PPc or AIx in participants with CLI, alternative indexes of aortic stiffness (PPc and AIx) were increased in participants with CLI; and AIx was well correlated with PPc in these participants. Thus, a decline in PWV in CLI is unlikely to be attributed to reductions in aortic stiffness. A feasable explanation for the decreased carotid-femoral PWV in CLI is that a decrease in distending pressures distal to arterial stenoses may result in an attenuation of stiffness in these segments. In this regard, the percentage stenosis of the carotid artery is correlated with an increased pulse wave transit time across this arterial bed (Horrocks *et al.* 1979). When considering possible explanations it is also important to reflect on the determinants of PWV as defined by the Moens-Korteweg equation (PWV = $\sqrt{[E \times h/2r\rho]}$), where E represents Young's modulus of a thin-walled homogeneous elastic tube, h is wall thickness, 2r the diameter of the lumen and ρ is the constant related to fluid density (O'Rourke *et al.* 2002). In this regard, in advanced atherosclerosis, compensatory enlargement (Glagov effect) of large vessels may occur which could increase 'r' and thus potentially decrease PWV. However, one would not expect compensatory enlargement to exceed the original diameter and hence this is an unlikely explanation. It is also possible that blunting of the upstroke of the femoral pressure waveform could lead to late foot detection, overestimation of the carotid-femoral transit time and underestimation of PWV. At this point there is no feature in SphygmoCor software that assesses the extent to which blunting of the upstroke of the femoral pulse wave occurs. These potential explanations require further investigation.

The results of this study are of potential clinical relevance, suggesting that a reduced carotid-femoral PWV in the context of an increased PPc may be employed to identify the

presence of advanced atherosclerosis. In this regard, I compared the relationships between a proposed stiffness mismatch index (PPc/PWV) and CLI against the relationships between a number of alternative easily and reliably obtained indexes of large artery disease (carotid IMT, AIx and PPc *per se*) and CLI. I noted that the relationship between the stiffness mismatch index and CLI was independent of alternative indexes of large artery disease and by far exceeded the capacity of these indexes to predict the presence of advanced PAD. This is particularly important considering the recognised utility of IMT measurements in predicting lower limb atheroma (Bots *et al.* 1997, Allan *et al.* 1997). Whether the proposed stiffness mismatch index can predict outcomes in patients with PAD or alternative cardiovascular diseases would require prospective evaluation. Given that as many as 55% of patients with CLI are asymptomatic in the 6 month period prior to surgery (Dormandy *et al.* 1994), and that current simple indexes of PAD such as ankle-brachial index although strong independent predictors of cardiovascular outcomes, have not been shown to predict CLI; the stiffness mismatch index may serve as a much needed easy and reproducible predictor of CLI.

A limitation of the present study is the cross-sectional design which provides no temporal information regarding the evolution of carotid-femoral PWV changes prior to the clinical manifestation of CLI. In this regard it is likely that carotid-femoral PWV increases well in advance of the development of PAD and begins to decrease as atherosclerosis becomes more advanced. Indeed, as reviewed (O'Rourke *et al.* 2002) there is considerable evidence to show that increases in carotid-femoral PWV predict outcomes beyond conventional risk factors. The present results do nevertheless urge for a cautious approach when using PWV values for routine risk prediction in that low-normal PWV values in the context of a high-normal PPc in high risk patients, could indicate the presence of advanced large artery disease which requires further investigation.

A second limitation of this study is that I did not assess carotid plaque scores in the community-based healthy participants as these were almost never observed. In this regard,

carotid plaque score may be a more sensitive indicator of the extent of atheroma formation than IMT measurements (Spence 2006). Whether the PPc/PWV mismatch index outperforms carotid plaque scores requires further evaluation.

A third limitation of this study is that the dominant ethnic group studied was of black African ancestry. Consequently, whether similar findings would be noted in other ethnic groups also requires further study.

In conclusion, the present study shows that in patients with CLI, i) despite increases in alternative indexes of aortic stiffness (PPc and AIx) and an index of atherosclerosis (IMT), carotid-femoral PWV is markedly attenuated.; ii) that direct correlations between carotid-femoral PWV and PPc (or AIx) are abolished and hence that a mismatch between stiffness indexes occurs; and iii) that a PPc/PWV stiffness mismatch index is associated with CLI independent of IMT, AIx or PPc *per se* and that this stiffness mismatch index is a strong independent predictor of CLI. I therefore propose a novel risk index (stiffness mismatch index) in PAD that requires prospective evaluation in both asymptomatic and symptomatic patients.

CHAPTER 3

Carotid Intima-Media Thickness in African Patients with Critical Lower Limb Ischemia Infected with the Human Immunodeficiency Virus.

Brand M, Woodiwiss AJ, Michel F, Booysens HL, Majane OHI, Maseko MJ, Veller MG, Norton GR. Carotid intima-media thickness in African patients with critical lower limb ischemia infected with the human immunodeficiency virus. J AIDS Clinic Res 2012;3(7):1-7.

Abstract

The extent to which human immunodeficiency virus (HIV) is associated with increases in carotid intima-media thickness (IMT) independent of conventional cardiovascular risk factors is unclear. Hence, I evaluated whether independent of conventional risk factors, an increased carotid IMT occurs in African HIV infected patients with chronic critical limb ischemia (CLI). Carotid IMT was measured in 217 sequentially recruited patients with CLI, 25 of whom were HIV positive and in 430 randomly selected controls from a community sample. As compared to HIV negative patients with CLI, HIV positive patients were younger (49±10 vs 64±11 years, $p<0.0001$) and had a markedly lower prevalence of hypertension and diabetes mellitus ($p<0.0001$), but a similar proportion of patients smoked (76% vs 67%). However, as compared to patients with CLI who were HIV negative, HIV positive patients had a similar increase in carotid IMT (HIV positive=0.75±0.14 mm; HIV negative=0.79±0.14 mm; Controls=0.64±0.15, $p<0.0001$ versus Controls) even after adjustments for age, sex and conventional risk factors (HIV positive=0.75.±0.13 mm; HIV negative=0.73±0.15 mm, Controls=0.66±0.15, $p<0.005$). IMT was similarly increased in HIV positive patients with CLI as compared to controls when assessed in men, smokers, and black African patients only ($p<0.05$-0.0001), or in those who were receiving highly active antiretroviral therapy (n=12, 0.74±0.10 mm) as compared to those not receiving therapy (0.75±0.15 mm). As compared to controls, the age- sex- and conventional risk factor-adjusted odds of having an IMT≥0.8 mm was similarly increased in patients with CLI who were HIV positive (odds ratio=8.89, CI=2.79-28.32, $p=0.0002$) as those who were HIV negative (odds ratio=2.70 CI=1.51-4.81, $p<0.001$). In conclusion, these results suggest that despite being of a younger age, with or without conventional risk factor adjustments, marked increases in carotid IMT in HIV in Africa are a risk factor for CLI.

3.0 Introduction

There is increasing evidence that infection with human immunodeficiency virus (HIV) is associated with occlusive arterial disease including myocardial infarction (Triant *et al.* 2007, Currier *et al*. 2003, Klein *et al*. 2002) and peripheral arterial disease (PAD) (Periard *et al.* 2008, Ye *et al.* 2010). A number of mechanisms may explain this relationship. Partly through antiretroviral therapy (specifically protease inhibitors)(Seminari *et al.* 2002), patients with HIV infections have an increased prevalence of conventional cardiovascular risk factors (van Vonderen *et al*. 2009). The consequence may be endothelial dysfunction (Hsue *et al*. 2004), and an enhanced degree of subclinical atherosclerosis as indexed by increases in carotid intima-media thickness (IMT) (Hsue *et al.* 2004, Grunfeld *et al*. 2009, Hulten *et al*. 2009). However, vascular pathology in HIV may also occur through the virus infecting the arterial vasculature (Tabib *et al.* 1992, Bobryshev *et al*. 2000), and increasing the chances of vascular inflammatory changes occurring either directly (Hsue *et al.* 2009) or through co-infections (Sacre et al. 2012, Hsue *et al* . 2006, Lemström *et al.* 1995). Thus, increases in carotid IMT in HIV infected patients may not only index the degree of atherosclerosis, but also alternative large artery phenotypes (Spence 2006). The measurement of carotid IMT may therefore be useful at predicting cardiovascular risk beyond conventional risk factors in HIV infected patients. Presently however there is considerable uncertainty as to whether increases in carotid IMT occur in HIV independent of conventional risk factors.

A recent meta-analysis (Hulten *et al.* 2009) and a large study (Grunfeld *et al*. 2009) not included in this meta-analysis (Hulten *et al*. 2009) have suggested that the conventional risk factor-independent effects of HIV or antiretroviral therapy on common carotid IMT are modest and that to some extent these positive relationships reflect a publication bias (Hulten *et al*. 2009). Thus, increases in carotid IMT previously reported on in HIV (Hsue *et al*. 2004,

Grunfeld C *et al.* 2009, Hulten *et al*. 2009) are more likely to be attributed to atherosclerosis mediated by conventional risk factors. However, there are no studies that have explored the relationship between HIV and carotid IMT in patients with clinical manifestations of advanced occlusive arterial disease. In this regard, recent studies have indicated that HIV infected patients in Africa presenting with occlusive arterial disease may have fewer risk factors for atherosclerosis, and angiographically or at surgery they are more frequently noted to have a lower atherosclerotic burden (Mulaudzi *et al*. 2005, Becker *et al.* 2010). Consequently, in the present study, I aimed to compare the extent to which IMT is increased independent of conventional risk factors in African patients with chronic critical lower limb ischemia (CLI) who are HIV infected as compared to those without evidence of HIV.

3.1 Methods

3.1.1 Study groups.

Details of the selection of study groups have been outlined in chapter 2, section 2.1.1. Routine HIV serology (ELISA) was performed to determine HIV status and 25 patients with CLI were identified as having an HIV infection. Data obtained in patients with CLI were compared with data acquired in 430 participants older than 16 years, from randomly recruited nuclear families of black African descent with at least two parents or two siblings and living in the South West Township (SOWETO) of Johannesburg, South Africa who had carotid IMT measurements (Norton *et al*. 2012). In this regard, the Chris Hani Baragwanath Hospital serves the SOWETO community and patients attending the Charlotte Maxeke Johannesburg Academic Hospital are generally from a similar socioeconomic class and ethnic group as those living in the SOWETO community.

3.1.2 Demographic and clinical data.

As described in chapter 2, section 2.1.2, a questionnaire was administered to obtain demographic information including each participant's medical history, the use of medication and tobacco and alcohol use. Obesity was defined as a body mass index (BMI) $\geq$30 kg/m^2. Blood tests were performed including a fasting lipid profile and glycated haemoglobin (HbA_{1C}). A CD4 count was obtained in all HIV positive patients with CLI. Participants were considered to have diabetes mellitus if they had a fasting plasma glucose concentration $\geq$7mmol/l, or in whom glucose-lowering agents were prescribed. Brachial blood pressure (BP) was measured according to guidelines and taken as the mean of five measurements. Participants with a BP$\geq$140/90 mm Hg or in those receiving antihypertensive medication were considered to have hypertension. As all patients with CLI were receiving statins, dyslipidaemia was diagnosed as the presence of either a raised triglyceride concentration ($\geq$1.7 mmol/l) or a reduced high density lipoprotein (HDL) concentration (<1.0 mmol/l for males and <1.2 mmol/l for females).

3.1.3 Carotid intima-media thickness (IMT).

Carotid IMT was determined as described in section 2.1.3 using high resolution B-mode ultrasound (SonoCalc IMT, Sonosite Inc, Bothell, Washington).

3.1.4 Data analysis.

Database management and statistical analysis was performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC). To determine differences in IMT multiple

regression analysis was performed using a general linear model and the adjusted means (least squared means) were compared. To identify the conventional risk factors for CLI in HIV infected or HIV negative patients, and to determine the relationship between increased carotid IMT and CLI multivariate logistic regression analysis was performed. Included in the multivariate models were age, sex, hypertension, diabetes mellitus, smoking, triglyceride concentrations and HDL cholesterol concentrations. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood as implemented by the mixed procedure as defined in the SAS package. As patients with CLI with an HIV infection were younger than those without evidence of an HIV infection, sensitivity analysis was conducted in HIV negative patients with CLI that were <60 years of age. As more men than women had CLI, the majority of patients with CLI smoked, in comparisons against the community sample, sensitivity analysis was also conducted in men only and smokers only. As HIV infected patients were of black African descent, whilst a proportion of HIV negative patients with CLI were white or Asian, sensitivity analysis was also conducted in black Africans only. Except for proportions, all data are shown as mean±SD.

3.2 Results

3.2.1 Participant characteristics.

The demographic and clinical characteristics of the participants are shown in Table 3.1. More men than women were noted to have CLI. As compared to randomly selected healthy participants from the community sample, patients with CLI who were HIV negative were older, fewer were of black African ancestry, and a higher prevalence of traditional risk factors, except for obesity, the prevalence of which was lower, was noted (Table 3.1).

Table 3.1. Characteristics of participants.

	Community sample	Critical lower limb ischemia	
		HIV infection	
		Yes	No
Sample size	430	25	192
% Males	35.3	84.0	69.3
Age (years)	44±18	49±10*††	64±11*
Black African (%)	100	92	59
Body mass index (kg/m^2)	29.5±8.3	23.8±4.2*	26.3±5.2*
Waist circumference (cm)	90.6±18.1	77.1±14.6*	85.8±17.1*
% Obese	41.4	8.3*	19.3*
% Hypertensive	44.7	16.0*†	67.7*
% Receiving antihypertensives	26.1	16.0*†	67.7*
% With diabetes mellitus	9.1	8.0†	49.5*
% Receiving glucose-lowering agents	6.3	4.0	10.9
% Receiving lipid-lowering agents	0	100	100
Current smoker (%)	15.8	76.0*	66.7*
Regular alcohol (%)	22.8	68.0*	55.2*
Systolic blood pressure (mm Hg)	128±22	129±20†.	137±21*
Diastolic blood pressure (mm Hg)	83±13	79±12.	80±12
Glycated hemoglobin (%)	6.05±1.29	5.95±0.51	7.74±2.56*
Total/HDL cholesterol	3.49±1.17	3.86±1.29	4.06±1.74
LDL cholesterol (mmol/l)	2.65±0.90	2.32±0.79	2.16±0.99
HDL cholesterol (mmol/l)	1.41±0.45	1.20±0.44	1.09±0.46*
Triglycerides (mmol/l)	1.16±0.66	1.47±0.78*	1.35±0.64*
CD4 count (x10^6/l)	-	325±122	-

HIV, human immundeficiency virus. * p<0.0001 versus community sample; † p<05, ††p<0.001 versus no evidence of HIV.

Whilst receiving statins, serum HDL cholesterol concentrations were markedly lower and triglyceride concentrations higher in HIV negative patients with CLI (Table 3.1).

More patients with as compared to without an HIV infection with CLI were of black African ancestry (Table 3.1). Patients with CLI infected with HIV had a markedly lower prevalence of traditional risk factors as compared to HIV negative patients with CLI, except for smoking, the prevalence of which was similar in HIV infected patients with CLI (Table 3.1). As compared to healthy participants, patients with CLI infected with HIV had a significantly lower prevalence of hypertension and obesity and more patients smoked (Table 3.1). However, the prevalence of diabetes mellitus was similar to the control group (Table 3.1). As compared to the control group, whilst receiving statins, patients with CLI infected with HIV had higher triglyceride concentrations, although the mean values were not higher than accepted international thresholds (Table 3.1). No differences in triglyceride concentrations were noted between the HIV infected and the HIV negative patients with CLI.

The characteristics of the 12 HIV infected patients receiving highly active antiretroviral therapy (HAART) were similar to those not receiving therapy (Table 3.2).). In the clinics at Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Hospital, Johannesburg, South Africa, the current HAART first-line therapy consists of 3 agents, namely 2 nucleoside reverse transcriptase inhibitors and 1 non-nucleoside reverse transcriptase inhibitor. None of the patients were receiving protease inhibitors.

Table 3.2. Characteristics of treated (HAART) versus non-treated (non-HAART) HIVpositive patients with critical limb ischemia.

	HAART	non-HAART
Sample size	12	13
% Males	83.3	84.6
Age (years)	46±8	52±11
Black African (%)	100	84.6
Body mass index (kg/m^2)	23.0±5.0	24.5±3.1
% Obese	8.3	7.7
% Hypertensive	8.3	23.1
% With diabetes mellitus	8.3	7.7
Current smoker (%)	75.0	69.0
Regular alcohol (%)	75.0	61.5
Systolic blood pressure (mm Hg)	130±20	129±21
Diastolic blood pressure (mm Hg)	82±10	76±13
Glycated hemoglobin (%)	5.95±0.30	5.98±0.97
Total/HDL cholesterol	3.35±1.00	4.15±1.30
LDL cholesterol (mmol/l)	2.10±0.73	2.53±0.83
HDL cholesterol (mmol/l)	1.36±0.53	1.06±0.27
Triglycerides (mmol/l)	1.47±0.73	1.46±0.85
CD4 count (x10^6/l)	334±95	316±149
Carotid IMT (mm)	0.74±0.10	0.76±0.14
Duration of treatment (years)	1.2±0.9 (range 0.3 to 1.5)	-

HIV, human immunodeficiency virus; IMT, intima-media thickness.

3.2.2 Comparison of conventional risk factors between patients with CLI <60 years without evidence of an HIV infection.

As compared to patients with CLI and HIV infection (Table 3.1), a greater proportion of HIV negative patients with CLI <60 years of age (n=64, mean age=51.4±7.2 years, p=0.31 versus patients with CLI and an HIV infection) had hypertension (60.9%, p<0.0005) and diabetes mellitus (56.3%, p<0.0001). As compared to patients with CLI and an HIV infection (Table 3.1) a similar proportion of HIV negative patients with CLI <60 years of age smoked (56.3%, p=0.23).

3.2.3 Conventional cardiovascular risk factors associated with CLI in patients with versus without evidence of HIV infection.

Table 3.3 shows the unadjusted and age- and sex-adjusted odds of conventional cardiovascular risk factors increasing the chances of CLI. As compared to the control group, in patients with HIV infection, smoking, and dyslipidaemia as defined by reduced HDL cholesterol concentrations were the only conventional risk factors associated with CLI. In contrast, in HIV negative patients, irrespective of whether or not they were <60 years of age; smoking, diabetes mellitus, hypertension and dyslipidaemia as defined by raised triglyceride concentrations (in patients <60 years of age) and a low HDL cholesterol concentration were all associated with CLI. The odds of hypertension being associated with CLI was reduced in patients with an HIV infection as compared to patients without evidence of an HIV infection.

Table 3.3. Conventional cardiovascular risk factors associated with critical limb ischemia (CLI) in patients with (+)(n=25) or without (-)(n=192) a human immunodeficiency virus (HIV) infection as compared to a community sample (n=430).

		Odds ratios (OR) (95% Confidence intervals)			
CLI vs	Subgroup (n)	Unadjusted	p value	Adjusted*	p value
Smoking	HIV- (192)	10.70 (7.16-15.83)	<0.0001	10.12 (6.01-17.08)	<0.0001
	HIV-<60 yrs (64)	10.90 (6.09-19.52)	<0.0001	7.75 (4.12-14.55)	<0.0001
	HIV-≥60 yrs (128)	10.52 (6.72-16.49)	<0.0001	12.84 (6.74-24.47)	<0.0001
	HIV+ (25)	16.86 (6.50-43.75)	<0.0001	10.87 (3.96-29.78)	<0.0001
Hypertension	HIV- (192)	2.60 (1.82-3.72)	<0.0001	0.76 (0.47-1.21)	=0.50
	HIV-<60 yrs (64)	1.93 (1.13-3.31)	<0.05	1.15 (0.62-2.13)	=0.06
	HIV-≥60 yrs (128)	3.05 (1.99-4.69)	<0.0001	0.44 (0.25-0.79)	<0.05
	HIV+ (25)	0.24 (0.08-0.70)†	<0.0001	0.12 (0.04-0.40)†	<0.01
DM	HIV- (192)	9.82 (6.36-15.16)	<0.0001	6.71 (4.0-11.3)	<0.0001
	HIV-<60 yrs (64)	12.89 (7.12-23.53)	<0.0001	15.06 (7.41-30.60)	<0.0001
	HIV-≥60 yrs (128)	8.57 (5.31-13.84)	<0.0001	3.93 (2.10-7.14)	<0.0001
	HIV+ (25)	0.87 (0.20-3.84)†	=0.86	0.95 (0.20-4.54)	=0.60
Increased TG	HIV- (192)	1.56 (1.03-2.37)	<0.05	0.88 (0.54-1.43)	=0.59
	HIV-<60 yrs (64)	2.47 (1.38-4.43)	<0.005	1.91 (1.02-3.58)	<0.05
	HIV-≥60 yrs (128)	1.19 (0.72-1.98)	=0.49	0.47 (0.26-0.86)	<0.05
	HIV+ (25)	0.69 (0.20-2.37)	=0.55	0.53 (0.15-1.92)	=0.28
Decreased	HIV- (192)	3.17 (2.18-4.59)	<0.0001	3.73 (2.34-5.95)	<0.0001
HDL cholest	HIV-<60 yrs (64)	4.37 (2.50-7.63)	<0.0001	5.81 (3.11-10.86)	<0.0001
	HIV-≥60 yrs (128)	2.69 (1.76-4.13)	<0.0001	2.66 (1.54-4.60)	=0.001
	HIV+ (25)	2.75 (1.17-6.47)	<0.05	3.76 (1.51-9.36)	<0.01

DM, diabetes mellitus; TG, tryglycerides; HDL cholest, high density lipoprotein fraction of cholesterol. *Age and sex-adjusted. Probability values were further adjusted for non-independence of family members. Increased TG indicates values ≥1.7 mmol/l. Decreased HDL cholesterol indicates values<1.0 mmol/l for males and <1.2 mmol/l for females. †p<0.001 vs OR for HIV- group.

3.2.4 Carotid intima-media thickness.

Table 3.4 shows unadjusted, and multivariate adjusted carotid IMT values. Without or with age- and sex-adjustments or age-, sex-, and conventional risk factor- adjustments, carotid IMT was markedly increased in both the HIV infected and HIV negative patients with CLI. Sensitivity analysis conducted in HIV negative patients with CLI <60 years of age, men only, smokers only and in black Africans only, showed similar differences as noted in all participants. Furthermore, multivariate adjusted IMT values in HIV positive patients with CLI who were <60 years of age (0.73±0.12 mm) who had a mean±SD age of 45±6 years were higher than multivariate adjusted IMT values in all participants from the community sample (0.64±0.11 mm, $p<0.0005$) who had a mean±SD age of 44±18 years. Importantly, no differences in IMT were noted in patients with CLI with as compared to without evidence of an HIV infection. No differences were noted in the carotid IMT in patients receiving HAART (n=12, 0.74±0.10 mm) as compared to those not receiving HAART (0.75±0.15 mm).

3.2.5 An increased carotid intima-media thickness as a risk factor for CLI in HIV infected versus HIV negative patients.

Table 3.5 shows the unadjusted; sex- and age-adjusted; and sex-, age- and conventional risk factor-adjusted odds of having an increased carotid IMT in patients with CLI as compared to controls in HIV infected and HIV negative patients. As compared to controls the presence of an increased carotid IMT increased the odds of having CLI irrespective of whether patients were HIV infected or HIV negative. As compared to controls, the odds of having an increased IMT in patients with CLI who were HIV infected were similar to those who were not infected with HIV. In sensitivity analysis these results were consistent.

Table 3.4. Comparisons of unadjusted and multivariate-adjusted carotid intima-media thickness values in patients with critical limb ischemia with (+) or without (-) a human immunodeficiency virus infection and in apparently healthy participants from a community sample.

Adjustments	Community sample	CLI HIV infection Yes	CLI HIV infection No	p-values community sample vs HIV+	p-values community sample vs HIV-
		All participants			
Sample size	n=430	n=25	n=192		
Unadjusted	0.63±0.14	0.75±0.14	0.79±0.14	<0.0001	<0.0001
Age- and sex-	0.66±0.12	0.75±0.13	0.73±0.14	<0.0005	<0.0001
Age-, sex- and other*	0.66±0.15	0.75±0.13	0.73±0.15	<0.0005	=0.0001
		HIV negative patients with CLI<60 years old			
Sample size	n=430	n=25	n=64		
Unadjusted	0.63±0.14	0.75±0.14	0.79±0.14	<0.0001	<0.0001
Age and sex-adjusted	0.64±0.12	0.73±0.12	0.75±0.12	<0.0005	<0.0001
Age-, sex- and other*	0.64±0.12	0.72±0.13	0.76±0.15	<0.0005	<0.0001
		Men			
Sample size	n=152	n=21	n=133		
Unadjusted	0.64±0.16	0.75±0.14	0.79±0.14	<0.005	<0.0001
Age- and sex-	0.68±0.16	0.75±0.13	0.73±0.14	=0.017	<0.005
Age-, sex- and other*	0.68±0.17	0.76±0.15	0.75±0.17	=0.014	<0.05
		Smokers			
Sample size	n=68	n=19	n=128		
Unadjusted	0.63±0.14	0.76±0.14	0.80±0.14	<0.0005	<0.0001
Age- and sex-	0.66±0.16	0.77±0.14	0.78±0.15	<0.005	<0.0001
Age-, sex- and other*	0.66±0.17	0.77±0.15	0.78±0.16	<0.005	<0.0001
		Black Africans			
Sample size	n=430	n=23	n=113		
Unadjusted	0.63±0.14	0.75±0.14	0.79±0.14	<0.0001	<0.0001
Age- and sex-	0.66±0.12	0.75±0.12	0.71±0.13	<0.0001	<0.0005
Age-, sex- and other*	0.65±0.12	0.75±0.13	0.72±0.15	<0.0005	<0.0005

*Additional adjustments are for hypertension, diabetes mellitus, smoking (except for analysis conducted in smokers only), triglyceride concentrations and HDL cholesterol concentrations. Probability values were further adjusted for non-independence of family members. No differences were noted in groups with or without HIV infection.

Table 3.5. Relationship between an increased carotid intima-media thickness (IMT≥0.8 mm) with critical limb ischemia in patients with (+)(n=25) or without (-)(n=192) a human immunodeficiency virus infection as compared to a community sample (n=430).

Carotid IMT≥0.8 vs	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
			All participants			
Adjustments	Unadjusted		Age- and sex		Age-, sex- and conventional risk factors*	
CLI in patients HIV-	6.34 (4.18-9.61)	<0.0001	2.89 (1.79-4.66)	<0.0001	2.70 (1.51-4.81)	<0.0001
HIV+	7.52 (3.24-17.44)	<0.0001	7.69 (2.92-20.25)	<0.0001	8.89 (2.79-28.32)	<0.0001
			HIV negative patients with CLI<60 years old			
Adjustments	Unadjusted		Age-, and sex-		Age-, sex-, and conventional risk factors*	
CLI in patients HIV-	5.95 (3.33-10.63)	<0.0001	5.16 (2.64-10.09)	<0.0001	4.77 (1.91-11.88)	<0.0001
			Men			
Adjustments	Unadjusted		Age-		Age-, and conventional risk factors*	
CLI in patients HIV-	4.82 (2.72-8.57)	<0.0001	2.73 (1.46-5.13)	<0.005	1.88 (0.80-4.46)	=0.15
HIV+	6.86 (2.59-18.15)	=0.0001	8.34 (2.81-24.76)	<0.0001	8.83 (2.10-37.21)	=0.001
			Smokers			
Adjustments	Unadjusted		Age, and sex-Age-, sex- and conventional risk factors (except smoking)*			
CLI in patients HIV-	10.12 (3.82-26.82)	<0.0001	6.69 (2.25-19.92)	<0.0005	5.03 (1.61-15.74)	<0.01
HIV+	14.00 (3.89-50.39)	<0.0001	8.77 (2.15-35.72)	<0.0005	11.29 (1.74-73.10)	<0.01
			Black African			
Adjustments	Unadjusted		Age, and sex-		Age-, sex- and conventional risk factors*	
CLI in patients HIV-	6.47 (4.01-10.44)	<0.0001	2.69 (1.56-4.66)	<0.001	2.96 (1.45-6.04)	<0.005
HIV+	7.47 (3.12-17.87)	<0.0001	9.00 (3.32-24.40)	<0.0001	9.90 (3.01-32.60)	<0.0001

OR, odds ratio; CI, confidence interval. See Table 4 for sample sizes in subgroups. *Additional adjustments are for hypertension, diabetes mellitus, smoking, triglyceride concentrations and HDL cholesterol concentrations. Probability values were further adjusted for non-independence of family members.

Furthermore, as compared to all participants from the community sample who had a mean±SD age of 44±18 years, an increased multivariate adjusted odds of having an increased carotid IMT in HIV positive patients with CLI who were <60 years of age and who had a mean±SD age of 45±6 years was 11.54 (confidence intervals=3.29-40.54, p<0.0001).

3.3 Discussion

The main findings of this study are as follows. As compared to patients with CLI who were HIV negative and had a number of conventional cardiovascular risk factors associated with CLI, patients with CLI who were HIV positive were approximately 15 years younger and other than smoking and dyslipidemia, had very few other conventional risk factors (8% diabetes mellitus and 16% hypertension) for atherosclerosis. Despite the lower age and conventional risk factor profiles in patients with CLI who were HIV positive as compared to those who were HIV negative, patients with CLI who were HIV positive had similar unadjusted and age- and sex-adjusted increases in carotid IMT or the odds of having an increased IMT as compared to randomly selected participants from a community sample. With further adjustments for alternative conventional risk factors, patients with CLI infected with HIV retained as high a carotid IMT and odds of an increased IMT as HIV negative patients with CLI. Moreover, in sensitivity analysis the increased carotid IMT and odds of an increased IMT in HIV infected patients with CLI was consistent across a number of groups including men only, smokers only and those of black African descent. Furthermore, the increased IMT and odds of an increased IMT in HIV infected patients with CLI was comparable with the increased IMT and odds of an increased IMT in HIV negative patients with CLI <60 years of age. The use of HAART did not influence the extent of the increase in carotid IMT in patients with CLI infected with HIV.

Although previous studies have demonstrated that HIV is associated with an increased carotid IMT (Hsue *et al.* 2004, Grunfeld *et al.* 2009), the extent to which this occurs independent of conventional risk factors is controversial (Hulten *et al.* 2009). This study is the first to show that in advanced occlusive arterial disease, such as occurs in CLI, HIV infected patients who are a distinct group both demographically (younger age) and with respect to the absence of important conventional cardiovascular risk factors (hypertension and diabetes mellitus) except smoking and dyslipidemia, have as extensive an increase in common carotid IMT as patients with CLI who are HIV negative. Even with adjustments for age, smoking and the presence of dyslipidaemia, the increases in carotid IMT in patients with CLI and HIV infection were equivalent to that observed in HIV negative patients with CLI. As triglyceride concentrations were similarly increased in the HIV positive and negative patients with CLI, it is unlikely that the increased carotid IMT in HIV positive patients could be attributed to co-infection-induced hypertriglyceridaemia. In addition, as none of the HIV infected patients were receiving protease inhibitors, the use of protease inhibitors could not account for the increased carotid IMT in the HIV positive patients with CLI. The relationship between carotid IMT and CLI in HIV infected patients in this study therefore suggests that despite being of a younger age, independent of conventional cardiovascular risk factors or protease inhibitor use, carotid IMT may serve as a strong risk factor for occlusive arterial disease in HIV.

The characteristics of the HIV positive patients with CLI in the present study were similar to those reported in previous studies describing the presence of occlusive arterial disease in HIV infected African patients (Mulaudzi *et al*. 2005, Becker *et al*. 2010). In this regard, as with these previous studies ((Mulaudzi *et al*. 2005, Becker *et al*. 2010), HIV positive patients with occlusive arterial disease were younger and had considerably less conventional risk factors other than smoking than HIV negative patients. In prior studies conducted in HIV positive patients with occlusive arterial disease, the burden of

atherosclerosis was considerably lower than HIV negative patients (Mulaudzi *et al*. 2005, Becker *et al*. 2010). Thus, in the present study it is also possible that the burden of atherosclerosis was considerably lower in the HIV positive as compared to the HIV negative patients with CLI. It is therefore unlikely that the similarity in the extent of the increase in carotid IMT in HIV positive as compared to negative patients can be attributed to a comparable degree of carotid atherosclerosis. Rather, the significant increase in carotid IMT in HIV positive patients is likely to reflect in-part a carotid phenotype other than typical atherosclerosis. In this regard, there is increasing evidence to support the view that carotid IMT is a measure of arterial phenotypes other than atherosclerosis (Spence 2006).

Possible explanations for increases in IMT beyond that which can be explained by atherosclerotic risk factors require consideration. In this regard, a vascular inflammatory process mediated by cytomegalovirus (CMV)-induced immune responses may occur in HIV (Sacre *et al.* 2012, Hsue *et al* .2006). Indeed, CMV has been demonstrated to produce intimal thickening in experimental studies (Lemström *et al.* 1995) and after controlling for CMV-specific immune responses, HIV-related increases in carotid IMT are abolished (Hsue *et al.* 2006). Alternatively, HIV may be associated with an increased arterial inflammatory infiltrate (Hsue *et al*. 2012) possibly mediated by increased concentrations of chemo-attractant proteins such as monocyte chemo-attractant protein 1 (Alonso-Villaverde *et al*. 2004). Moreover, co-infections with herpes simplex virus or Chlamydia pneumoniae (Libby *et al*. 1997) may also cause a vascular infiltrate.

In this study I evaluated common rather than internal carotid artery IMT. A previous large study provided evidence to show that as compared to data obtained in the common carotid artery, data obtained in the internal carotid artery shows a markedly greater conventional risk factor-independent effect of HIV on IMT (Grunfeld *et al*. 2009). Thus, the use of the common carotid artery may underestimate the quantitative effect of HIV

independent of conventional risk factors. The design of the present study nevertheless provides an inherent control for this limitation as I was able to compare IMT in the common carotid artery in HIV positive patients with CLI to that noted in patients with advanced occlusive arterial disease (CLI) who were HIV negative. In this regard, the extent of the increase in IMT in the common carotid was equivalent in the two groups.

When adjusting for the adverse effects of conventional cardiovascular risk factors on carotid IMT, I could not adjust for absolute blood pressure or low-density lipoprotein cholesterol concentrations as continuous traits as a high proportion of patients with CLI were receiving antihypertensive and lipid lowering therapy. Thus I cannot discount the possibility of residual confounding effects explaining the independent relationships between CLI and carotid IMT in HIV infected patients. In this regard however, the same limitation applies to the HIV negative group with CLI as applies to the HIV infected patients with CLI, and yet the carotid IMT values in both of these groups were similarly increased as compared to the control group. Hence, I believe that by studying a group of HIV negative patients with CLI, the chance of residual confounding effects produced by differences in blood pressure or lipid profiles is unlikely to have significantly contributed to the conventional risk factor-independent relations between CLI and carotid IMT in HIV infected patients.

In this study I noted that the majority (76%) of patients with CLI who were HIV infected were smokers. These data are consistent with the high prevalence of smoking in HIV infected individuals with occlusive arterial disease in previous reports (Klein *et al*. 2002, Becker *et al*. 2010, Saves *et al.* 2003, Friis-Moller *et al.* 2003). Smoking therefore represents an important confounder when assessing relationships between HIV and carotid IMT. Although I showed similar increases in carotid IMT in HIV infected patients with CLI as compared to those without evidence of HIV infection after adjustments for smoking and in sensitivity analysis conducted in smokers only, the duration of smoking could not be adjusted

for. However, as HIV infected patients with CLI were 15 years younger on average than HIV negative patients, it is more likely that those without HIV smoked for a longer duration and hence we are likely to have biased against the results of the present study.

The limitations of the present study are as follows. This was a cross-sectional study and hence no conclusions regarding cause and effect between HIV and carotid IMT can be drawn. It is therefore still possible that unidentified risk factors for carotid IMT other than the HIV infection could still account for the similarity in the increase in carotid IMT in patients with CLI infected as compared to uninfected with HIV. Only a longitudinal study will resolve this issue, but with the introduction of HAART as part of in routine clinical care in patients with HIV infections, a carefully controlled longitudinal study is not ethically feasible. Second, inherent in any study that assesses patients with advanced occlusive arterial disease indexed by the presence of CLI, the interpretation of the results of the present study are limited by the small number of patients with CLI identified as having an HIV infection (25 of 217 patients). However, the selection of sequential patients with CLI was necessary to attempt to avoid a selection bias. Moreover, the study sample reported on probably reflects as large a sample as one could expect given the advanced nature of the occlusive arterial disease studied and the overall prevalence of HIV. Importantly, the study was statistically powered to show the effects explored. Indeed I had a <1 to 1% chance of a type I error. As a consequence of the stigma linked to HIV infection in South Africa, I also could not perform tests of HIV status in the apparently healthy community sampled. Based on current trends of HIV prevalence in this community, I predict that approximately 15% of the community would be HIV infected (Ijsselmuiden *et al*. 1993). However, this would have biased against the results of the present study as the mean carotid IMT in the apparently healthy community may have been lower than that identified if we had excluded individuals with HIV infection. Last, I did not assess plaque scores or measures of plaque volume to assess the extent of the atherosclerotic burden

in the present study. This may have assisted in distinguishing between the atherosclerotic versus non-atherosclerotic carotid phenotype (Spence 2006).

In conclusion, I show that independent of conventional cardiovascular risk factors or HAART, IMT in the common carotid artery is increased to a similar extent in patients with CLI who are HIV positive as compared to those who are HIV negative. This was noted despite patients with CLI who were HIV infected being of a younger age (on average 15 years younger) and having fewer conventional risk factors for cardiovascular disease. In contrast to the high prevalence of hypertension and diabetes mellitus in HIV negative patients with CLI even in those <60 years of age; in those patients with CLI who were HIV positive neither hypertension, nor diabetes mellitus were risk factors for CLI with or without adjustments for sex and age. However, patients with CLI who were HIV positive did have as high a prevalence of smoking and possibly dyslipidaemia as HIV negative patients with CLI, but these factors were adjusted for in multivariate models. These results therefore suggest that an increased carotid IMT may be a strong independent risk factor for CLI in HIV infected African patients.

CHAPTER 4

Prevalence and Haemodynamic Characteristics of Asymptomatic Moderate-to-Severe Left Ventricular Systolic Dysfunction in Critical Lower Limb Ischaemia.

Abstract

Asymptomatic decreases in left ventricular (LV) ejection fraction (EF) predict long-term mortality and a decreased patency of endovascular interventions in patients undergoing vascular surgery. In patients with advanced peripheral arterial disease (PAD), the prevalence of asymptomatic moderate-to-severe decreases in EF and the associated haemodynamic disturbances are uncertain. Echocardiography was performed in 93 sequentially recruited patients with chronic critical lower limb ischaemia (CLI) without symptoms of heart failure and 698 randomly recruited participants from a community sample of whom 177 were age-matched. As compared to the age-matched control group from the community sample, patients with CLI had a significantly reduced multivariate adjusted EF (CLI=55.6±12.4%, Control=66.2±12.1%, $p<0.0001$), LV midwall fractional shortening (FSmid)($p<0.0001$), stroke volume index (SV)($p<0.0001$), cardiac output index (CO)($p<0.05$), and increased total peripheral resistance index (TPR)($p<0.05$). In contrast to none of the age-matched community participants, 26/93 (28%) patients with CLI had an EF <40%, of which only 7 had a previous myocardial infarction. In patients with CLI with an EF <40%; CO, SV and FSmid were all substantially reduced ($p<0.0001$), N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations and early-to-atrial transmitral velocity were increased ($p<0.05$), whilst LV end diastolic volume index was marginally increased ($p<0.05$) as compared to those with an EF ≥55%. In conclusion, CLI is associated with a high prevalence of asymptomatic moderate-to-severe reductions in EF, independent of coronary artery disease. This translates into a decreased CO and increased TPR, alterations that may contribute toward an increased mortality or reduced patency of endovascular interventions after vascular surgery in patients with CLI.

4.0 Introduction

Although the prognostic value of the presence of heart failure symptoms on postoperative outcomes is acknowledged in recent guidelines (Fleisher *et al.* 2007, Poldermans *et al.* 2009), the value of echocardiographic measures of left ventricular ejection fraction (EF) in predicting survival after surgery is controversial (Wijeysundera *et al.* 2011). Patients with peripheral arterial disease (PAD) requiring surgery are well recognised as being at risk for surgical outcomes due to the high prevalence of coexistent coronary artery disease (CAD)(Fawkes *et al*. 1992, Aronow *et al.* 1994) and heart failure (Anand *et al*. 2007, Conrad *et al.* 2006, Bakken *et al*. 2009, Apelqvist *et al.* 2011, O'Brien-Irr *et al*. 2011) and consequently heart failure associated with a reduced EF (Anand *et al.* 2007). However, in advanced PAD, where general mobility is limited, exertional dyspnoea, a major feature of heart failure, may not be a common complaint in those patients with cardiac abnormalities. In these circumstances echocardiographic evaluation of LV dysfunction may have prognostic value. Asymptomatic decreases in EF are associated with a greater risk of long-term cardiovascular mortality after non-cardiac open vascular surgery (hazards ratio=4.6) including surgery for PAD (Flu *et al.* 2010). Furthermore, the reduced patency of peripheral endovascular interventions associated with the presence of heart failure in PAD (Conrad *et al.* 2006, Bakken *et al*. 2009, Apelqvist *et al.* 2011, O'Brien-Irr *et al*. 2011) has largely been attributed to reduced left ventricular EF (Meltzer *et al.* 2012). An increased mortality or a reduced patency of peripheral endovascular interventions associated with reductions in EF (Flu *et al*. 2010, Meltzer *et al.* 2012) are likely to be attributed to haemodynamic disturbances, such as a reduced blood flow and marked vasoconstriction. However, as highlighted in chapter 1, the prevalence of asymptomatic left ventricular systolic dysfunction independent of clinical evidence of CAD and the haemodynamic disturbances that

characterize decreases in EF in unselected patients with PAD about to undergo surgery are uncertain.

In this study I aimed to evaluate the prevalence of asymptomatic left ventricular systolic dysfunction independent of clinical evidence of CAD and the haemodynamic disturbances that characterize decreases in EF in consecutive patients with chronic critical lower limb ischemia (CLI) requiring surgery.

4.1 Methods

4.1.1 Study groups.

Details of the selection of study groups have been outlines in chapter 2, section 2.1.1. In the present study 93 consecutive patients with CLI had echocardiography. Data obtained in patients with CLI were compared with data obtained in 177 age-matched healthy participants selected from a community-based sample held in the APOGH database. In this regard, the community sample consisted of 698 participants older than 16 years, randomly recruited from the South Western Township (SOWETO) of Johannesburg, South Africa (Norton *et al*. 2008, Libhaber *et al*. 2009) These participants were considered to be appropriate controls as the Chris Hani Baragwanath Hospital serves the SOWETO community and patients attending the Charlotte Maxeke Johannesburg Academic Hospital are generally from a similar socioeconomic class and ethnic group as those living in the SOWETO community. The 177 participants from the community-based study were selected as controls if they were of an age $\geq$ the lower 10% confidence interval for age of participants with CLI and had a BMI $\leq$ the upper 90% confidence interval for BMI of participants with CLI.

4.1.2 Demographic and clinical data.

As described in section 2.1.2, a questionnaire was administered to obtain demographic information including each participant's medical history, the use of medication and tobacco and alcohol use. Obesity was defined as a body mass index (BMI) $\geq$30 kg/m^2. Blood tests were performed including a fasting lipid profile, glycated haemoglobin (HbA_{1C}) and N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations (in patients with CLI only). Participants were considered to have diabetes mellitus if they had a fasting plasma glucose concentration ≥7mmol/l, or in whom glucose-lowering agents were prescribed. Brachial blood pressure (BP) was measured according to guidelines and taken as the mean of five measurements. Participants with a BP≥140/90 mm Hg or in those receiving antihypertensive medication were considered to have hypertension. NT-proBNP was used as a marker of heart failure (Maisel 2001), and a concentration $\geq$2000 pg/mL was considered to 'rule in' cardiac dysfunction (Maisel *et al.* 2011).

4.1.3 Echocardiography

Echocardiography was performed as previously described (Norton *et al.* 2008, Libhaber *et al.* 2009). Measurements were obtained by myself under the supervision of an experienced echocardiographer using either a pulse color Doppler Hewlett Packard model 4500-5500 recorder coupled to a 2.5 MHz transducer or a SonoSite version 3.4 model coupled to a 2.5-MHz transducer. Left ventricular (LV) dimensions were determined using two-dimensional directed M-mode echocardiography in the short axis view and these recordings analyzed according to the American Society of Echocardiography convention (Sahn *et al.* 1978). During recordings, the transducer was placed perpendicular to the chest

wall or pointed slightly inferiorly and laterally at the end of the long axis. All measurements were recorded on videotape and analyzed off-line by observers who were unaware of the clinical details of the participants. Figure 4.1 shows a representative M-mode image employed to assess left ventricular mass and function. The interventricular septal wall thickness (IVS) at end diastole and end systole, the posterior wall thickness (PWT) at end diastole and end systole and the end diastolic and end systolic internal dimensions of the left ventricle were measured only when appropriate visualization of both the right and the left septal surfaces occurred. Left ventricular diastolic function was assessed from a pulsed wave Doppler examination of the mitral inflow at rest (Nagueh *et al*. 2009). In this regard, early (E) and late (atrial-A) transmitral velocity was determined and diastolic function assessed from E/A ratios and the E wave deceleration time. All measurements were recorded and analyzed off-line by experienced investigators whom were unaware of the clinical data of the participants.

Left ventricular end diastolic and systolic volumes were determined using both the Teichholz (Teichholz *et al*. 1976) and the Z-derived (de Simone *et al*. 1996) methods. Left ventricular EF (biplane Simpson) and midwall fractional shortening (FSmid) were calculated to determine LV chamber and myocardial systolic function respectively using standard formulae. Left ventricular EF was determined as [(LV end diastolic volume-LV end systolic volume)/ LV end diastolic volume] x 100. A reduced EF was defined according to a variety of thresholds over the range of 40-55% in order that comparisons could be made with prior studies. However, a normal EF was considered to be ≥55%. Midwall fractional shortening (FSmid) was calculated using a previously described formula (de Simone *et al*. 1994, de Simone *et al*. 2002) as [(LVIDed + 0.5 Hed)-(LVIDes + 0.5 Hes)]/(LVIDed + 0.5 Hed), where LVID is left ventricular internal diameter, H is wall thickness, ed is end diastole and es is end systole.

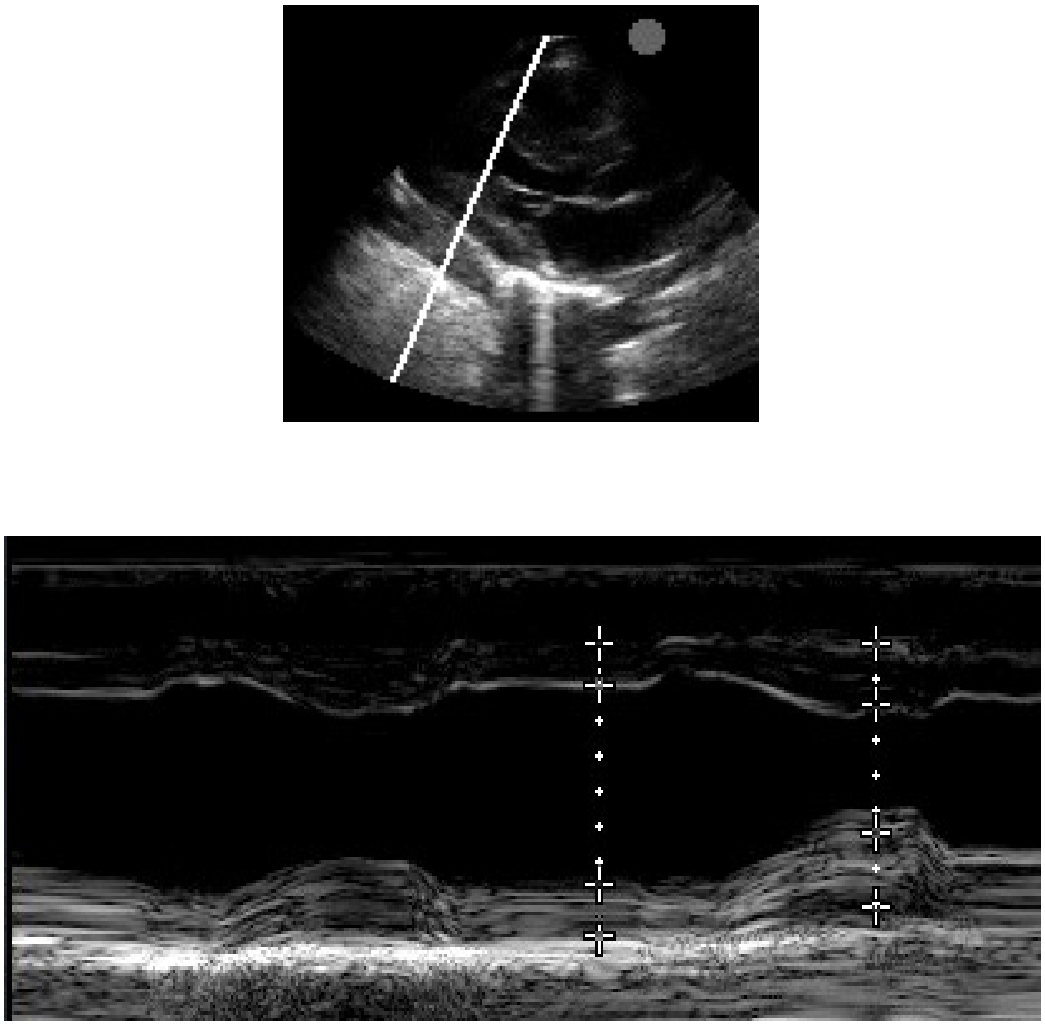

Figure 4.1. A two-dimensional guided (upper panel) M-mode echocardiographic image (lower panel) derived from a Hewlett Packard model 5500 utilised to assess left ventricular dimensions and function.

The calcution of FSmid using a modified ellipsoidal model and as previously described (de Simone *et al.* 1994) accounts for epicardial migration of the midwall during systole. Stroke volume was evaluated from the difference between LV end diastolic and systolic volumesdetermined using both the Z-derived method (de Simone *et al.* 1996) and indexed to body surface area. Cardiac output (CO) and total peripheral resistance (TPR) indices were determined from SV index x pulse rate and mean arterial pressures/CO index respectively. Circumferential LV systolic wall stress was calculated as previously described (Shimizu *et al.* 1991) as:

$$\frac{\text{SBP } (0.5\ \text{LVIDs})^2\ [1+ \{(0.5\ \text{LVIDs} + \text{PWTs})^2/(0.5\ \text{LVIDs} + 0.5\ \text{PWTs})^2\}]}{(0.5\ \text{LVIDs} + \text{PWTs})^2 - (0.5\ \text{LVIDs})^2}$$

where SBP is systolic blood pressure, LVIDs is LVID in systole and PWTs is posterior wall thickness in systole. Left ventricular mass was derived according to an anatomically validated formula (Devereux et al 1986) (LVM = 0.8 x [1.04 (LVEDD + IVS +PWT) ^{3} – (LVEDD) ^{3}] + 0.6g) and indexed to height$^{2.7}$ (LVM index, LVMI). Left ventricular relative wall thickness (RWT) was calculated as (LV diastolic posterior wall thickness x2)/LV end diastolic diameter (Ganau *et al.* 1992). Left ventricular mean wall thicklness was determined from the mean of LV septal and posterior wall thickness. Left ventricular hypertrophy (LVH) was defined as a LVMI >51 g/m$^{2.7}$ for both women and men (Nunez *et al.* 2005).

Intra-observer variability studies were conducted on 29 subjects on whom repeat echocardiographic measurements have been performed within a two week period of the initial measurements. The Pearson's correlation coefficients for LV end diastolic diameter, septal wall thickness and posterior wall thickness were 0.76, 0.94 and 0.89 (all $p<0.0001$) respectively, and the variances (mean % difference ± SD) were 0.12±5.95%, -0.77±4.47% and 0.67±5.57% respectively. In addition, no significant differences between repeat

measurements were evident on paired t-test analysis (p=0.99, p=0.42 and p=0.48 respectively).

4.1.4 Data analysis

Database management and statistical analyses were performed with SAS software, version 9.2 (SAS Institute Inc., Cary, NC, USA). Continuous data are reported as mean±SD. Unadjusted means and proportions were compared by the large-sample z-test and the χ^2-statistic, respectively. Independent relations were assessed from multivariate linear or logistic regression analysis with appropriate adjustors. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood as implemented by the mixed procedure as defined in the SAS package.

4.2 Results

4.2.1 Participants characteristics

The general characteristics of patients with CLI versus the community sample are shown in Table 4.1. A trend for a greater number of men than women and a decreased BMI was noted in the group with CLI. Considerably more participants with CLI had diabetes mellitus and smoked. Glycated haemoglobin concentrations were increased in the group with CLI. Although a similar proportion of participants with CLI had hypertension, more were receiving antihypertensive therapy. Consequently, blood pressures were lower in participants with CLI. Considerably more participants with CLI were receiving lipid-lowering agents and as a consequence lipid concentrations were lower in the group with CLI. Heart rate was

significantly increased in participants with CLI. 7.5% of patients with CLI had a previous myocardial

4.2.2 Echocardiographic measures in CLI versus controls

Echocardiographic measures in patients with CLI versus age-matched participants from the community sample are shown in Table 4.2. Patients with CLI had a reduced EF, stroke volume index, cardiac output index, and FSmid. These values persisted with adjustments for age, gender, diabetes mellitus, hypertension and a previous MI. Total peripheral resistance index was increased in patients with CLI after adjustments for potential confounders. However, neither LV end diastolic diameter, LV end diastolic volume index, nor LVMI were increased in patients with CLI as compared to the community sample. Patients with CLI had similar LV systolic stress values as controls. The decrease in EF was noted even after adjustments for LV systolic wall stress (EF, Community sample=66.2±12.1%, CLI=55.6±12.4%, $p<0.0001$). Patients with CLI had similar E/A and E wave deceleration times as patients from the community sample.

4.2.3 Prevalence of a reduced EF in participants with CLI versus controls.

No control participants had a moderate-to-severe reduction in EF. However, 26 of 93 (28%) participants with CLI had moderate-to-severe reductions in EF and of these participants only 7 had a history of MI. None of the participants with an EF <40% had electrocardiographic evidence of a previous MI.

Table 4.1. Characteristics of patients with critical lower limb ischemia and age-matched control participants from a community sample.

	Control (n=177)	CLI (n=93)
Age (years)	59.7±10.7	62.1±11.8
Male (n [%])	101 (57.1)	68 (73.1)*
Body mass index (kg/m^2)	27.1±3.6	26.1±9.9*
Obese (n [%])	46 (26.0)	15 (16.1)*
Smoking (%)	19.2	57.0***
Hypertension (n [%])	123 (69.5)	55 (59.1)
Treated for hypertension (n [%])	73 (41.2)	55 (59.1)**
Diabetes mellitus (n [%])	30 (17.0)	42 (45.2)***
Lipid lowering agents (n [%])	0 (0)	93 (100)***
Total cholesterol (mmol/l)	5.03±2.38	3.97±1.31***
LDL cholesterol (mmol/l)	2.86±0.96	2.16±1.02***
Total cholesterol/HDL ratio	3.92±1.33	4.16±1.62
HbA1c (%)	6.52±1.91	7.53±2.61**
Heart rate (beats/min)	65±14	83±17***
Systolic blood pressure (mm Hg)	142±23	132±16***
Diastolic blood pressure (mm Hg)	88±13	79±11***
Mean arterial pressure (mm Hg)	106±14	97±12***

BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; HbA1c, glycated haemoglobin. *p<0.05, **p<0.005, ***p<0.0001 vs control.infarction (MI) and no patients had angina pectoris. No participants from the community-based study had a history of cardiovascular events.

Table 4.2. Comparison of echocardiographic variables in patients with critical lower limb ischemia versus age-matched control participants from a community sample.

	Control (n=177)	CLI (n=93)
LV ejection fraction (%)	67.3±9.8	53.4±20.8**
Adjusted† ejection fraction (%)	66.1±14.1	55.8±14.6**
Stroke volume (mls)	65.2±17.6	47.4±17.8**
Stroke volume index (mls/m^2)	37.0±9.6	26.0±10.3**
Adjusted† stroke volume index (mls/m^2)	37.2±10.2	25.8±10.5**
Cardiac output (l/min)	4.20±1.26	3.95±1.7
Cardiac output index (l/min/m^2)	2.38±0.67	2.16±0.94*
Adjusted† cardiac output index (l.min.m^2)	2.40±0.81	2.12±0.83*
TPR index (mm Hg/l/min/m^2)	16.3±6.4	16.7±9.1
Adjusted† TPR index (mm Hg/l/min/m^2)	15.6±7.5	17.9±7.7*
LV wall stress (g/cm^2)	117±33	121±41
LV mass index (g/$m^{2.7}$)	45.4±14.9	39.3±13.2*
LV end diastolic diameter (cm)	4.80±0.58	4.32±0.71**
LV end diastolic volume index (mls/m^2)	55.1±13.4	46.8±14.5**
LV mean wall thickness (mm)	0.94±0.21	1.07±0.16**
LV relative wall thickness	0.40±0.09	0.49±0.10**
LV FSmid (%)	22.5±6.1	16.3±9.0**
Adjusted† LV FSmid (%)	22.0±7.5	17.2±7.7**
E/A	1.02±0.34	1.03±0.53
E wave deceleration time (ms)	223±62	213±63

LV, left ventricle; TPR, total peripheral resistance; FSmid, fractional midwall shortening; E, early transmitral velocity; A, transmitral velocity during atrial contraction. *$p<0.05$, **$p<0.0001$ versus control. †Adjustments are for age, gender, diabetes mellitus, hypertension and myocardial infarction. p-values were further adjusted for non-independence of family members.

4.2.4 Echocardiographic features of participants with CLI with a reduced ejection fraction.

The clinical and echocardiographic characteristics of patients with CLI with an EF <40% as compared to those with an EF ≥55% are shown in Table 4.3. As compared to patients with CLI with an EF ≥55%, more patients with CLI with an EF <40% had a previous MI. However, a similar proportion were hypertensive or diabetic and a similar proportion were receiving antihypertensive medication. As compared to patients with CLI with an EF ≥55%, patients with CLI with an EF <40% had a reduced cardiac output index and FSmid and an increased total peripheral resistance index. These differences were noted with adjustments for MI. Although LV end diastolic diameter and LV end diastolic volume index were increased in those with an EF <40% as compared to those with an EF ≥55%, only 19.2% (5/26) of patients had an LVEDD >5.5 cm, 11.5% (3/26) an LV end diastolic volume index >75 ml/m^2 and LVEDD and LV end diastolic volume index in participants with an EF <40% was similar to LVEDD and LV end diastolic volume index as healthy participants from the general population (Table 4.3 versus Table 4.2). Patients with CLI and an EF <40% had a similar LVMI, E wave deceleration time as compared to those with an EF ≥55%. However, patients with CLI and an EF <40% had increased E/A and NT-proBNP concentrations as compared to patients with CLI and an EF ≥55%.

Table 4.3. Demographic, clinical and echocardiographic characteristics of patients with critical lower limb ischemia with and without a left ventricular ejection fraction <40%.

	EF <40% (n=26)	EF ≥55% (n=53)
Age (years)	61±13	62±10
Male (n [%])	19 (73)	38 (72)
Hypertension (n [%])	18 (69)	31 (59)
Diabetes mellitus (n [%])	14 (54)	22 (42)
Myocardial infarction (n [%])	7 (27)*	0 (0)
Heart rate (beats/minute)	81±13	83±18
Systolic blood pressure (mm Hg)	134±16	132±17
LV ejection fraction (%)	25.2±11.7**	68.2±8.2
Adjusted† LV ejection fraction (%)	25.9±11.8**	67.9±10.2
Cardiac output index (l/min.m^2)	1.52±0.64**	2.53±0.97
Adjusted† cardiac output index (l/min.m^2)	1.36±0.74**	2.61±0.87
TPR index (mm Hg/l/min/m^2)	22.5±11.7**	14.0±7.0
Adjusted† TPR index (mm Hg/l/min/m^2)	25.1±11.6**	12.7±6.4
LV mass index (g/$m^{2.7}$)	44.3±14.0	38.3±13.1
LV FSmid (%)	6±5**	22±7
Adjusted† LV FSmid (%)	6±5**	22±7
LV end diastolic diameter (cm)	4.66±0.87*	4.20±0.64
LV end diastolic volume index (mls/m^2)	53.1±17.2*	44.9±13.6
E/A	1.35±0.74*	0.91±0.36
NT-Pro brain natriuretic peptide (pg/ml)	2015±4263*	464±678

LV, left ventricle; TPR, total peripheral resistance; FSmid, fractional midwall shortening; E, early transmitral velocity; A, transmitral velocity during atrial contraction. *p<0.05, **p<0.0001 vs EF≥55%. †Adjustments are for myocardial infarction.

4.2.5 Sensitivity and specificity of increased proBNP concentrations for the identification of a reduced ejection fraction.

Using a proBNP level of >2000 pg/ml as predictive of heart failure (Maisel *et al.* 2011), the sensitivity, specificity, positive predictive value and negative predictive value of NT-proBNP for an EF <40% was 42.9%, 67.9%, 25% and 82.6% respectively.

4.3 Discussion

The main findings of the present study are as follows: The presence of CLI was associated with a high prevalence of moderate-to-severe decreases in EF (<40%) independent of the accompanying symptoms or signs of heart failure, clinical evidence of CAD or additional confounders. The decrease in EF was associated with decreases in stroke volume and cardiac output indexes and an increased total peripheral resistance index. The decrease in EF was mainly attributed to an attenuated myocardial systolic function (decreased FSmid), despite similar systolic stress values (afterload) and after adjustments for heart rate. The decrease in EF was only minimally related to adverse LV chamber remodelling in that LV end diastolic diameter and volume indexes were only marginally increased in patients with an EF <40% as compared to those with an EF ≥55%, but these values were no greater than participants from a community sample and only 12% and 19% had an LV end diastolic volume index or an LV end diastolic diameter respectively that could be considered to be increased.

Although prior studies indicate that PAD is independently associated with CAD (Fawkes *et al.* 1992, Aronow *et al.* 1994) and heart failure (Anand *et al.* 2007, Conrad *et al.* 2006, Bakken *et al.* 2009, Apelqvist *et al.* 2011, O'Brien-Irr *et al.* 2011) the extent to which

asymptomatic moderate-to-severe left ventricular systolic chamber dysfunction occurs in unselected consecutive patients with PAD requiring surgery is uncertain. Previous studies conducted in select clinical samples have reported wide prevalence rates of a reduced EF, and it is uncertain to what extent this was associated with symptoms of heart failure or clinical evidence of CAD. A prevalence rate of 15-24% of patients with PAD requiring surgery with an EF <35-50% has previously been reported (Rossi *et al*. 1998, Shrikhande *et al*. 2007, Flu *et al*. 2010, van Kuijk *et al*. 2010, Iida *et al*. 2012, Table 1.3). However, in these studies 33-89% of patients had CAD (Rossi *et al*. 1998, Shrikhande *et al.* 2007, Flu *et al*. 2010, van Kuijk *et al*. 2010, Iida *et al*. 2012, Table 1.3), in three studies 20-67% of participants had a prior history of heart failure (van Kuijk *et al*. 2010, Shrikhande *et al*. 2007, Franco *et al*. 1989) and in one study 57% had a history of heart disease (Iida *et al*. 2012). In contrast to these studies (Rossi *et al*. 1998, Shrikhande *et al*. 2007,Flu *et al*. 2010, van Kuijk *et al*. 2010, Iida *et al*. 2012, Table 1.3), in this study I noted 28% of patients with CLI with an EF <40% and an absence of heart failure symptoms, 27% had a history or clinical evidence of CAD, and none had a prior history of heart failure. In contrast to this and previous (Rossi *et al.* 1998, Shrikhande *et al.* 2007, Flu *et al*. 2010, van Kuijk *et al*. 2010, Iida *et al.* 2012, Table 1.3) studies, one previous study reported a prevalence of an EF<50% as being 89% (Franco et al. 1989). There is no overt reason for the discrepancy between this (Franco *et al*. 1989) and the present or prior (Rossi *et al*. 1998, Shrikhande *et al.* 2007,Flu *et al*. 2010, van Kuijk *et al.* 2010, Iida *et al*. 2012, Table 1.3) studies.

An explanation for the relationship between a decreased EF and a reduced patency of endovascular interventions in patients with heart failure (Meltzer *et al*. 2012) is that systolic dysfunction may be associated with an attenuated peripheral blood flow, an increased total peripheral resistance and endothelial or vascular smooth muscle cell changes (Kubo *et al.* 1991, Drexler *et al*. 1992, Ledoux *et al*. 2003). In this study I show decreases in cardiac

output and increases in total peripheral resistance in patients with CLI and asymptomatic decreases in EF. It is therefore possible that in these patients, a similar risk for a reduced patency of endovascular interventions may exist. A decreased EF without symptoms in patients with CLI, as noted in the present study, may also increase long-term cardiovascular mortality after open vascular surgery (Flu *et al*. 2010). The present findings therefore support prospective or intervention studies being conducted evaluating the impact of asymptomatic reductions in EF or the effect of medical therapy on these reductions in EF, on outcomes in CLI. If outcomes are in-part predicted by asymptomatic decreases in EF, this may provide additional guidance as to management approaches in patients with CLI. In this regard, a reduced EF may support the use of endovascular as opposed to open vascular surgery in patients without symptoms of heart failure (Flu *et al*. 2010), or encourage practitioners to attempt to improve EF and potentially blood flow using medical therapy such as angiotensin-converting enzyme inhibitors and beta-blockers.

The decreases in stroke volume and cardiac output indexes in patients with CLI with asymptomatic reductions in EF are at apparent odds with prior work demonstrating that the difference between symptomatic and asymptomatic decreases in EF is the haemodynamic effects which are noted in these patients (Konstam *et al.* 1992). In this regard, as compared to symptomatic decreases in EF, asymptomatic decreases in EF have previously been reported to be associated with sustained stroke volumes produced through increases in preload (increases in LV end diastolic volumes) (Konstam *et al.* 1992). In this study, although patients with a reduced EF had increased LV end diastolic diameters and volume indices as compared to those with an EF ≥40%, these values did not exceed values in healthy participants from the community study and only 19% had LV end diastolic diameters greater than 5.5 cm and 12% had a LV end diastolic volume index greater than 75 ml/m^2. Thus, I assume that the reductions in stroke volume and cardiac output in patients with an EF <40% are in-part

attributed to an inability to appropriately increase LV preload in response to a decrease in myocardial systolic function. The mechanisms responsible for this effect could be through restriction of cardiac filling as E/A was markedly increased in patients with an EF <40% as compared to patients with an EF ≥55%.

In this study the reductions in EF in patients with CLI independent of clinical evidence of CAD were associated with decreases in FSmid. As FSmid is a measure of LV systolic function independent of chamber geometry, it is possible that the mechanism responsible for the decreases in EF is mainly through an attenuation of LV contractility. The decreases in EF and FSmid were independent of hypertension or diabetes mellitus, and hence could be a consequence of underlying asymptomatic CAD. Whether current medical therapy advocated for use in heart failure can improve EF when the principle cause is a reduced myocardial contractility rather than adverse LV remodelling, would require evaluation.

The limitations of the present study include the following. This was a cross-sectional study and hence I cannot draw conclusions with respect to cause-effect relationships. Furthermore, I assessed LV function using M-mode imaging and calculated stroke volume from short axis dimensions measurements using previously described and validated formulae (Teichholz *et al.* 1976, de Simone *et al.* 1996). However, this approach may be inaccurate, as it assumes an elliptical rather than a spherical LV shape, whilst in a dilated LV the heart becomes more spherical. Nevertheless, in the present study, LV dilatation was not a striking feature of the reduced EF in patients with CLI. The approach to LV volume estimations employed in the present study (Teichholz *et al.* 1976, de Simone *et al.* 1996) may also be limited by wall motion abnormalities in patients with MI. However, in the present study only 7 of 26 patients with an EF<40% had a previous MI. Therefore, it is unlikely that LV shape changes from previous MI would have contributed to any great extent to the reduced stroke volume and cardiac output indices in patients with CLI.

In conclusion, this study indicates that in the absence of symptoms or signs of heart failure, 28% of patients with CLI may have moderate-to-severe reductions in EF (<40%) and only a small proportion of these patients may have a history of pre-existing CAD. The decreased EF is associated with a reduced cardiac output and an increase in total peripheral resistance, alterations that may contribute toward adverse outcomes in patients with CLI.

CHAPTER 5

Summary and Conclusions.

5.1 Introduction

In my thesis I explored some potential novel approaches to risk assessment for advanced PAD. In this regard, I considered two broad issues. I have argued in favour of attempting to better identify those at risk of developing CLI, the end stage of PAD, that is associated with in a high risk of limb loss or death. I have adopted two approaches to identifying those at risk of CLI. In the first instance, I argue in favour of employing a large artery stiffness mismatch index for the identification of those who may ultimately develop CLI. Secondly, I suggest that in those patients who are HIV positive, the use of carotid intima-media thickness measurements may be particularly useful for identifying those at risk of developing CLI.

The second broad issue that I have considered is how best to approach that patient with CLI who has a high risk of death or limb loss. In this regard, because patients with CLI have a high prevalence of coronary artery disease and as such are at risk of cardiac systolic dysfunction, I have proposed that identifying those with LV systolic dysfunction may better stratify those at risk of a worse outcome attributed to death or a reduced patency of endovascular interventions. I evaluated whether patients requiring surgery because of CLI may indeed have a high prevalence of a reduced cardiac systolic function and the haemodynamic disturbances that could place them at risk of death of a reduced patency of endovascular interventions.

In this chapter I will summarise the findings of the aforementioned studies assessing possible novel risk approaches to advanced PAD. I will do so in the context of our current understanding of risk assessment in advanced PAD with suggestions as to how the current findings may be used to develop studies that build on current knowledge. I will discuss the findings of my thesis within the two broad issues presented in the preceding discussion, i.e.,

could the findings of the present thesis be further developed to improve the sensitivity of detecting those at risk of CLI, or those at risk of a poor outcome once CLI has developed? In this chapter I will also pose additional questions which arise from the findings of the present study, questions which require further assessment in follow-up studies to the studies conducted in this thesis. Further, I will discuss the potential limitations of the studies conducted in the present thesis and indicate how these limitations may be addressed in future studies.

5.2 Improving the sensitivity for risk detection in CLI

In the following section I will discuss the implications of the findings of the current thesis with respect to risk detection. Both the possibility of improving the sensitivity to detect those at risk of CLI and the possibility of detecting those at risk of a poor perioperative outcome in those requiring surgery for CLI will be considered.

5.2.1 Improving the sensitivity of detecting those generally at risk of CLI

Ultimately, the management of patients with CLI is not curative, but rather palliative (Norgren *et al.* 2007). There is therefore an urgent need for an easy and reliable method that will identify patients that may progress to CLI and prevent them from doing so. As emphasized in chapter 1, although symptomatic PAD (intermittent claudication-IC) is a strong risk factor for the development of CLI with 20-30% of claudicants ultimately requiring a revascularization procedure (Mccaslin *et al.* 2007, Mahoney *et al.* 2010), claudicants have a low risk of limb loss with only 2% developing CLI (Hirsch *et al.* 2006). Moreover, in a prospective multicentre study in patients requiring an amputation due to irreversible

ischaemia resulting from CLI, 55% of these patients were asymptomatic (no claudication) during the six months preceding their amputation (Dormandy *et al*. 1994). Hence IC is not an obligate risk factor for CLI. Ankle-brachial index measurements may provide some degree of ability to identify patients with CLI who will require amputation (Marston *et al*. 2006). Indeed, in a series of 142 patients (169 limbs) with CLI who did not undergo revascularization, the 12-month amputation rate for limbs with an ABI of 0.5-0.7 was l5%, for limbs with an ABI < 0.5, 32%, and for limbs with an ABI < 0.4, 43% (Marston *et al*. 2006). However, this approach does not allow for the identification of those who are likely to develop CLI.

In the present thesis, by studying sequentially recruited patients admitted to tertiary care hospitals with CLI, and using healthy participants randomly recruited from a community sample (the hospitals where patients with CLI were recruited from serve this community) as a comparator group, I show that age-related increases in aortic PWV are markedly attenuated in patients with CLI. This decrease in aortic PWV in patients with CLI occurred despite increases in alternative indexes of aortic stiffness including PPc and AIx and although the strong relationship between aortic PWV and PPc was lost in CLI, the relationship between AIx was well correlated with PPc in these participants. Thus, a decline in PWV in CLI is unlikely to be attributed to reductions in aortic stiffness. What is the explanation for these changes in aortic PWV, and how could this change be employed to identify those at risk of a CLI?

A potential explanation for the decreased carotid-femoral PWV in CLI may be present that there is a decrease in distending pressures distal to arterial stenosis that may result in an attenuation of stiffness in these segments. In this regard, the percentage stenosis of the carotid artery is correlated with an increased pulse wave transit time across this arterial bed (Horrocks *et al*. 1979). Pressure drops caused by local stenoses of carotid arteries of the order of 80

mmHg have been reported (Roberts *et al.* 1973). These decreases in pressure distal to the stenoses would lead to a tripling of the transit time in the artery distal to the stenosis (King *et al.* 1972). Could the changes in aortic PWV in the context of alternative changes in indexes of aortic stiffness be employed to detect those at risk of CLI?

In this thesis I evaluated whether a reduced aortic PWV in the context of an increased PPc, as evaluated using a proposed stiffness mismatch index (PPc/PWV), may predict CLI independent of or better than alternative indexes of large vessel abnormalities. I noted that the relationship between the stiffness mismatch index and CLI was independent of alternative indexes of large artery disease and far exceeded the capacity of these indexes to predict the presence of advanced PAD. This is particularly important considering the recognised usefulnee of IMT measurements in predicting lower limb atheroma (Bots *et al*.1997, Allan *et al.* 1997). Nevertheless, these associations were assessed in a cross-sectional study and would require evaluation in prospective studies to determine whether the stiffness mismatch index outperforms alternative risk factor assessments in predicting the development of CLI. In this regard, studies conducted in high-risk groups would be required, including in patients who both smoke and have diabetes mellitus. Therefore a longitudinal study is planned by our group. As will be highlighted in the subsequent section on potential limitations of the findings of this thesis (section 5.4), the outcomes of such a study are critical as it is possible that increases in the stiffness mismatch index index occur only late in the development of advanced PAD, and thus will only alert the physician to the potential development of CLI at a point where standard interventions are unable to prevent the onset of CLI. With respect to who should be followed up prospectively to evaluate the possible utility of the stiffness mismatch index, it is also important that a longitudinal study is conducted in those with IC to determine whether ABI and the stiffness mismatch index could be used in a complementary fashion.

5.2.2 Improving the sensitivity of detecting the HIV positive patient at risk of CLI

As highlighted in chapter 1, there is increasing evidence that infection with HIV is associated with occlusive arterial disease including PAD (Periard *et al.* 2008, Ye *et al.* 2010, Qaqa *et al.* 2011, Giannarelli *et al.* 2011). Between 20 and 26.5% of HIV positive patients have a decreased ABI (Periard *et al.* 2008, Qaqa *et al.* 2011). Human immunodeficiency virus infected patients may develop PAD, often in the absence of traditional risk factors and generally 20 years earlier than HIV negative patients (Periard *et al.* 2008, Giannarelli *et al.* 2011). As with HIV negative patients at risk of CLI, there is currently no predictor that heralds the onset of CLI in HIV positive patients. However, as non-traditional risk assessment of advanced PAD, which include measures of large artery structure and function, depend on effects on vascular remodelling, before considering the potential measurements that may predict CLI in HIV positive patients, it is important to recapitulate the mechanisms of CLI in HIV. In other words, it is important to consider whether one can expect the same changes in arterial structure and function in HIV infected patients with CLI as that which one would expect to occur in HIV negative patients with CLI.

A number of mechanisms may explain the relationship between HIV infection and PAD. Partly through antiretroviral therapy (specifically protease inhibitors), patients with HIV infections have an increased prevalence of conventional cardiovascular risk factors (van Vonderen *et al.* 2009). The consequence may be endothelial dysfunction (Hsue et al. 2004, Klein et al. 2005), and an enhanced degree of subclinical atherosclerosis as indexed by increases in carotid intima-media thickness (IMT) (Hsue *et al.* 2004, Micieli *et al.* 2007, Gutiérrez *et al.* 2008, Grunfeld *et al.* 2009, Hulten *et al.* 2009, van Vonderen *et al.* 2009). However, as emphasised in chapter 1, vascular pathology in HIV may also occur through HIV

infection *per se* (Kwiatkowska *et al*. 2011), an effect potentially mediated through the virus infecting the arterial vasculature (Tabib *et al*. 1992, Bobryshev *et al*. 2000), and increasing the chances of vascular inflammatory changes occurring either directly (Hsue *et al*. 2009), or through co-infections with for example cytomegalovirus, herpes simplex virus or Chlamydia pneumoniae (Lemström *et al*. 1995, Libby *et al*. 1997, Hsue et al. 2006, Sacre *et al*. 2012) which may influence plaque stability. Thus, changes in large artery structure or function in HIV infected patients may not only index the degree of atherosclerosis, but also alternative large artery phenotypes (Spence 2006). The measurement of large artery structure and function may therefore be particularly useful at predicting cardiovascular risk beyond conventional risk factors in HIV infected patients. Presently however there is considerable uncertainty as to whether increases in carotid IMT occur in HIV independent of conventional risk factors.

As explored in chapter 1, a recent meta-analysis (Hulten *et al*. 2009) and a large study (Grunfeld *et al*. 2009) not included in this prior meta-analysis (Hulten *et al*. 2009) have suggested that the conventional risk factor-independent effects of HIV or antiretroviral therapy on common carotid IMT are modest and that to some extent these positive relationships reflect a publication bias (Hulten *et al*. 2009). In a further meta-analysis described in chapter 1 with a number of additional studies included in the analysis, I have provided evidence to corroborate the outcomes of the meta-analysis conducted by Hulten *et al*. (2009). It is therefore possible that changes in large artery structure provide no measure beyond that determined from traditional risk factors of the development of PAD in HIV positive patients. In this thesis I therefore explored whether carotid IMT is increased to the same extent in HIV positive as in negative patients with CLI.

As compared to HIV negative patients with CLI, HIV positive patients with CLI had fewer traditional risk factors for atherosclerotic disease than HIV negative patients with CLI.

They were considerably younger (a 15 year age difference was noted), and other than smoking, HIV positive patients with CLI had no other conventional risk factors for atherosclerosis. Remarkably, despite their favorable risk factor profile, HIV positive patients had similar age- and sex-adjusted increases in carotid IMT or odds of having an increased IMT as compared to randomly selected participants from a community sample, as did HIV negative patients with CLI. Moreover, HIV positive patients with CLI retained as high a carotid IMT, and odds of an increased IMT, as HIV negative patients with CLI even following adjustment for smoking and the presence of dyslipidaemia. Treatment with HAART did not influence the extent of the increase in carotid IMT in HIV positive patients with CLI. The relationship between carotid IMT and CLI in HIV positive patients in the present study therefore suggests that independent of conventional cardiovascular risk factors or ART, carotid IMT may serve as a strong risk factor for peripheral occlusive arterial disease.

This study suggests that a study should be conducted in which patients with HIV with an increased carotid IMT should be identified and followed prospectively in an attempt to assess whether increases in IMT (such as those noted in the present study) are able to predict the development of CLI or cardiovascular events in these patients. Such a study may provide evidence for cardiovascular risk prediction beyond conventional risk factors and subsequently contribute toward the identification of those HIV infected patients most at risk of occlusive arterial disease.

5.2.3. Improving the sensitivity to detect the patient with CLI at risk of poor peri-operative outcomes.

As highlighted in chapter 1, PAD is often associated with CAD (Fawkes *et al*. 1992, Aronow *et al.* 1994) and as a consequence with heart failure (Anand *et al*.2007, Conrad *et al.*

2006, Bakken *et al.* 2009, Apelqvist *et al.* 2011, O'Brien-Irr *et al.* 2011). However, as also alluded to in chapter 1, in advanced PAD, where general mobility is limited, exertional dyspnoea, a major feature of heart failure, may not be a common complaint in those patients with cardiac abnormalities. Nevertheless, heart failure in advanced PAD is often associated with a reduced EF (Anand *et al.* 2007), and in the absence of heart failure symptoms, echocardiographic evaluation of LV EF may therefore have prognostic value. Although the prognostic value of the presence of heart failure symptoms on postoperative outcomes is acknowledged by recent guidelines (Fleisher *et al.* 2007, Poldermans *et al.* 2009), the value of echocardiographic measures of left ventricular EF in predicting survival after surgery is controversial (Wijeysundera *et al.* 2011). However, asymptomatic decreases in EF are associated with a greater risk of long-term cardiovascular mortality after non-cardiac open vascular surgery (hazards ratio=4.6) including surgery for PAD (Flu *et al.* 2010). Moreover, the reduced patency of peripheral endovascular interventions associated with the presence of heart failure in PAD (Conrad *et al.* 2006, Bakken *et al.* 2009, Apelqvist *et al.* 2011, O'Brien-Irr *et al.* 2011) has in part attributed to a reduced left ventricular EF (Meltzer *et al.* 2012). An increased mortality or a reduced patency of peripheral endovascular interventions associated with reductions in EF (Flu *et al.* 2010, Meltzer *et al.* 2012) are likely to be attributed to haemodynamic disturbances, such as a reduced blood flow and marked vasoconstriction. However, as highlighted in chapter 1, the prevalence of asymptomatic left ventricular systolic dysfunction independent of clinical evidence of CAD and the haemodynamic disturbances that characterize decreases in EF in unselected patients with PAD about to undergo surgery are uncertain.

In this thesis I demonstrated that CLI was associated with a high prevalence (28%) of moderate-to-severe decreases in EF (<40%) independent of the accompanying symptoms or signs of heart failure, clinical evidence of CAD (only 7.5% had a prior MI) or additional

confounders. The decrease in EF was associated with decreases in stroke volume and cardiac output indices and an increased total peripheral resistance index. These results provide evidence of the prevalence of asymptomatic moderate-to-severe decreases in EF in CLI with limited clinical evidence of CAD, and that these decreases in EF are associated with haemodynamic changes that one would expect to translate into an increased perioperative mortality or reduced patency of endovascular interventions. Prevalence rates of 15-24% of patients with PAD requiring surgery with an EF <35-50% have previously been reported (Rossi *et al.* 1998, Shrikhande *et al*. 2007,Flu *et al.* 2010, van Kuijk *et al*. 2010, Iida *et al*. 2012, Table 1.3), however, in these studies 33-89% of patients had CAD (Rossi *et al*. 1998, Shrikhande *et al*. 2007,Flu *et al.* 2010, van Kuijk *et al.* 2010, Iida *et al*. 2012, Table 1.3), in three studies 20-67% of participants had a prior history of heart failure (van Kuijk *et al*. 2010, Shrikhande *et al.* 2007, Franco *et al*. 1989) and in one study 57% had a history of heart disease (Iida *et al*. 2012).

The findings of a high incidence of asymptomatic moderate-to-severe reductions in EF in patients with CLI, and the associated decreases in cardiac output or increases in total peripheral resistance raises a number of questions. First, are these patients really asymptomatic or are they not reporting symptoms that may indicate potential underlying cardiac dysfunction. If this is the case, it is possible that a more systematic approach to detecting heart failure symptoms may be required in these patients to ensure that heart failure is not missed. Second, the results of the present study raise the possibility that a significant number of patients with CLI may be at a higher risk for adverse perioperative outcomes than previously thought. Further studies are required to ascertain whether these patients are indeed at a higher risk than expected. Moreover, exactly how to detect these patients and whether their detection would alter surgical decision-making processes would require prospective studies. Third, as the results of this study suggest that a significantly higher number of

patients with CLI have asymptomatic LV systolic dysfunction than expected, one wonders whether interventions which produce beneficial effects on the heart (eg: neurohumoral blockers) should not be used more frequently in patients with CLI. To answer this question, prospective, intervention studies are also required.

5.3 Additional questions raised by the findings of the present thesis

In addition to broaching the possibility of a number of novel assessments possibly predicting the risk of CLI in patients at a high cardiovascular risk or with HIV or identifying novel approaches to perioperative risk assessment in patients with CLI, the results of this thesis raise a number of additional issues which warrant further investigation.

5.3.1 Should carotid-femoral pulse wave velocity as a general risk predictor?

Current guidelines recommend the use of carotid-femoral PWV measurements for risk prediction beyond traditional risk factors (O'Rourke *et al*. 2002, Mancia *et al*. 2007). The results of this study raises the question of the use of carotid-femoral PWV for risk prediction, at least in asymptomatic cases of advanced atheroma. In this regard, alternative indices of arterial stiffness such as PPc or AIx may be considered for risk prediction under these circumstances. As I did not assess carotid-femoral PWV in less advanced forms of atheroma, and arterial stenoses may occur without symptoms, I also need to consider the possibility that carotid-femoral PWV may be an insensitive predictor of risk in less advanced forms of PAD, even in asymptomatic patients. This possibility however, will depend entirely on whether reductions in carotid-femoral PWV in PAD are only a late change, and don't occur in less advanced forms of PAD. In this regard, a number of further studies are required to evaluate

whether in the context of increases in PPc or AIx in cardiovascular disease or in high risk patients, carotid-femoral PWV does not increase to the extent that one would expect. It is important that studies are conducted to evaluate whether a mismatch between carotid femoral PWV and alternative indices of aortic stiffness do not occur even with modest reductions of ABI.

5.3.2 Does a reduced carotid-femoral pulse wave velocity in CLI reflect a decrease in aortic stiffness?

Although an unlikely possibility, the mismatch between aortic stiffness indices noted in CLI in the present study also raises the question of whether increases in aortic stiffness do occur in less advanced forms of PAD. In this regard, it is possible that increases in PPc and AIx in the patients with CLI may occur as a consequence of enhanced wave reflections attributed to changes in reflection points or the magnitude of wave reflection, whilst aortic stiffness may decrease. The decrease in aortic PWV noted in patients with CLI is reminiscent of the variable effects of diseases associated with advanced atheroma on aortic PWV. In this regard, as described in chapter 1, in experimental studies, atheroma formation has been associated with either an increased or reduced aortic PWV (Rutherford *et al.* 1997, Farrar *et al.* 1991). Moreover, patients with marked hypercholesterolaemia may exhibit an increased rather than reduced large artery compliance, and thus a reduced PWV (Dart *et al.* 1991, Lehmann *et al.* 1992, Bots *et al.* 1997, Allan *et al.* 1997). In addition, PWV decreases in SLE (Lee *et al.* 2006), a disease process often associated with extensive atheroma.

The increased aortic compliance previously reported on in patients with marked hypercholesterolemia at a high risk of atherosclerosis, but who are symptom free (Dart *et al.* 1991, Lehmann *et al.* 1992) has been attributed to the result of case selection of patients with

a low susceptibility to vascular "sclerosis" (Lehmann *et al*. 1991). Although the same argument could apply to the present study, selection against patients with "sclerosis" would be in favour of the development of CLI, and hence would suggest that the absence of "sclerosis" favours the development of CLI. This notion is contrary to the well-described "sclerotic" large artery changes that occur in advanced atherosclerotic disease (Stary *et al*. 1995) that accompanies CLI. It is nevertheless obvious that the mechanisms of the reductions in carotid-femoral PWV reported on in CLI in this thesis need further investigation. In this regard, as invasive, or computer assisted tomography/magnetic resonance imaging-angiography of the aorta or illiac arteries is infrequently available in South African hospitals, I could not assess the relationship between carotid-femoral PWV and the radius of aortic or iliac arteries. A study that specifically evaluates these relationships will nevertheless be required.

5.3.3 What does an increase in carotid IMT in HIV positive patients with CLI represent?

As highlighted in chapter 1, current practise in surgery departments in South Africa in patients with CLI and HIV is to perform thrombectomies rather than to place stents *in situ*. This has been based largely on the clinical impression that the vasculature is unaffected by the atherosclerotic process in HIV positive patients. Although the results of the present study do not challenge this approach, the present thesis nevertheless highlights the need to establish once and for all whether HIV-related vascular changes in occlusive arterial disease represent atherosclerotic abnormalities or a non-atherosclerotic phenotype. In this regard, there is increasing evidence to support the view that carotid IMT is a measure of arterial phenotypes other than atherosclerosis (Spence 2006).

In the present study, the traditional risk factor burden was considerably lower in the HIV positive as compared to the HIV negative patients with CLI, one has to consider the possibility that the similarity in the extent of the increase in carotid IMT in HIV positive as compared to negative patients may not be attributed to a comparable degree of carotid atherosclerosis. As IMT is approximately comprised on of 80% media and 20% intima, it may be more poorly correlated with factors that underlie the development of coronary artery lesions compared with other disease end points (Adams *et al.* 1995). Simon *et al.* (1998) noted a closer relationship between IMT and left ventricular mass than with coronary arterial disease, suggesting that IMT may reflect hypertensive medial hypertrophy (Spence 2004). However, in the present study the HIV positive group were significantly less hypertensive than the HIV negative group. Consequently this hypothesis may not apply to this study. The increase in carotid IMT in HIV positive patients could therefore reflect in-part a carotid phenotype other than typical atherosclerosis and a change which may not reflect a predominantly medial change. What are the possible factors that could explain a non-atherosclerotic phenotype in HIV that may not be attributed to predominant medial hypertrophy?

As enumerated in preceding sections, potential HIV-related changes that may increase carotid IMT include a vascular inflammatory process mediated by HIV infection (Lichtenstein et al. 2010), or opportunistic infectors such as cytomegalovirus (Hsue *et al.* 2006, Sacre *et al.* 2012), herpes simplex virus or Chlamydia pneumoniae (Libby *et al.* 1997). Cytomegalovirus has been demonstrated to produce intimal thickening in experimental studies (Lemström *et al.* 1995) and after controlling for CMV-specific immune responses, HIV-related increases in carotid IMT are abolished (Hsue *et al.* 2006). Alternatively, HIV may be associated with an increased arterial inflammatory infiltrate (Hsue *et al.* 2009) possibly mediated by increased concentrations of chemo-attractant proteins such as monocyte

chemo-attractant protein 1 (Alonso-Villaverde *et al.* 2004). Importantly, identifying the major cause of HIV-related increases in IMT may lend insights into the most appropriate therapeutic approach that should be used in patients with HIV and PAD. In this regard, I have initiated a study designed to assess the vascular changes that occur in HIV-positive patients with HIV infections. In this study I will be evaluating the vascular changes that occur in biopsy tissue obtained at surgery. This study nevertheless goes beyond the scope of the present thesis.

5.3.4 Traditional risk factors and CLI in HIV positive patients

In this thesis, though my focus was on attempting to identify novel approaches to risk assessment in patients with HIV and CLI, I did note that smoking was a major risk factor (76% were smokers) for CLI in patients with HIV infections. These data are consistent with the previously reported high prevalence of smoking in HIV positive individuals with occlusive arterial disease (Klein *et al.* 2002, Becker et al 2010, Savès *et al*. 2003). Importantly in one study assessing IMT in HIV infections, smoking was highly correlated with IMT (correlation co-efficient 0.42) (Kwiatkowska *et al.* 2011). Smoking therefore represents an important target for preventing occlusive arterial disease, in HIV.

5.4. Addressing the limitations of this thesis

The limitations of this thesis have largely been acknowledged in chapters 2-4 and in preceding sections of the present chapter, but it is worth recapitulating some of these limitations and indicating how future studies may address these points.

As discussed in all chapters, this was a cross-sectional study and hence I cannot draw conclusions with respect to cause-effect relationships. It is therefore possible that the

associations between CLI and decreases in carotid-femoral PWV (chapter 2), increases in IMT in HIV positive patients (chapter 3) and decreases in EF and CO (chapter 4) may not be attributed to CLI, HIV in CLI, or vascular pathology (CHD) associated with CLI. These findings therefore require substantiation in longitudinal studies or intervention studies where possible (e.g. assessing carotid-femoral PWV before and after an iliac artery endovascular intervention in CLI; the use of HAART in asymptomatic HIV patients with markedly increased IMT values; and the use of heart failure therapy in CLI patients with asymptomatic decreases in EF). The reasons for conducting cross-sectional studies in this thesis are as follows.

Both longitudinal and intervention studies will require considerable resources and time to complete and are not worth the investment without evidence from cross-sectional studies to support such studies. Following on a number of confirmatory studies, including the evaluation of aortic or iliac diameter measurements in patients with CLI; the assessment of the characteristic changes in the vasculature of patients with CLI and HIV; the evaluation of plaque scores and internal carotid IMT in patients with CLI and HIV, all of which have been discussed in previous sections, this thesis nevertheless the evidence to indicate that such longitudinal and intervention studies could be worthwhile.

The issue of sample size is always worthy of consideration in any study. The results of the present thesis were obtained from a reasonably large clinical population (217 patients with CLI) and from a large community-based study (1030 participants) conducted in a sample representative of the majority of patients recruited in this study. As such robust differences in indices of aortic stiffness were observed and these differences were noted across a wide range of ages and in sensitivity analysis conducted in specific and relevant subgroups. Although the number of patients with CLI who were HIV positive was small (n=25), the IMT changes noted in this group as compared to controls was sufficiently large that these differences

cannot be considered to reflect a type I statistical error. Of note is that these differences were also noted in relevant subgroups. Although the number of patients with CLI whom had echocardiographic measurements should also be considered as small (n=93), the EF changes were also sufficiently large that these differences cannot be considered to reflect a type I statistical error. However, in comparison to healthy volunteers, the changes in CO and TPR noted in patients with CLI were not as robust and hence these differences may be considered to reflect a type I statistical error. Nevertheless, patients with CLI with as compared to those without decreases in EF had changes in CO and TPR that cannot be considered to reflect a type I error.

A limitation of this thesis that also requires further consideration is the use of primarily one ethnic group to conduct the study. Although a lack of multiethnic groups may be relevant at an international level, as the majority of South Africans are of black African ancestry, the present study is of specific relevnce to South Africa.

5.5 Conclusions

In conclusion, the results of this thesis suggest that a decrease in carotid-femoral PWV in the context of an increase in PPc and AIx may herald the onset of CLI; that marked increases in carotid IMT may predict the potential for occlusive arterial disease in HIV and that asymptomatic reductions in EF with co-existent decreases in CO may accompany CLI. These results suggest novel potential approaches for risk prediction in advanced PAD.

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