PERIPHERAL ARTERIAL DISEASE AMONGST MINORITY ETHNIC GROUPS IN THE UNITED KINGDOM

by

PHILIP CHRISTOPHER BENNETT

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ABSTRACT

Peripheral arterial disease (PAD), a common manifestation of atherosclerosis, is an important healthcare problem with considerable morbidity and mortality. Intermittent claudication (IC) is the commonest symptomatic manifestation of this disease. This thesis investigates the prevalence of PAD in South Asians (people originating from India, Pakistan and Bangladesh) and Blacks (Black African, Black Caribbean) and makes ethnic comparisons of its associations with traditional cardiovascular risk factors, inflammatory and haemostatic biomarkers and markers of angiogenesis in these ethnic groups. This thesis also makes associations between common carotid intima media thickness, a marker of pre-clinical atherosclerosis, traditional cardiovascular risk factors and novel biomarkers. Furthermore this thesis validates translated versions of the Edinburgh Claudication Questionnaire into Punjabi and Urdu.

DECLARATION

The candidate confirms that the record of work submitted is his own and was carried out at the Haemostasis, Thrombosis and Vascular Biology Unit, The University Department of Medicine, City Hospital, Birmingham, UK.

I confirm that the work of the thesis and the writing of it were carried out entirely by me. I am thankful to my colleagues who offered assistance and co-operation towards the thesis. Appropriate nominal credit has been given, where reference has been made to the work of other scientists.

The work has not previously been accepted for a higher degree. A list of publications and papers presented to learned societies arising from work contained in this thesis is given at the end of this thesis.

Philip Christopher BENNETT

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GLOSSARY OF TERMS

AA	African American
AAA	Abdominal Aortic Aneurysm
ABPI	Ankle Brachial Pressure Index
ADP	Adenosine Diphosphate
AIx	Aortic Augmentation Index
AL	Andrew Lovick
Ang-1	Angiopoietin-1
Ang-2	Angiopoietin-2
As	Asian
Apo(a)	Apolipoprotein (a)
BMI	Body Mass Index
CAD	Coronary Artery Disease
CAM	Cellular Adhesion Molecule
CBVD	Cerebrovascular Disease
CCIMT	Common Carotid Intima Media Thickness
CEC	Circulating Endothelial Cell
Ch	Chinese
CI	Confidence Interval
CIMT	Carotid Intima Media Thickness
CLI	Critical Limb Ischaemia
CRP	C - Reactive Protein
CTA	Computerised Tomographic Angiography

CVD	Cardiovascular Disease
DM	Diabetes Mellitus
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
ECQ	Edinburgh Claudication Questionnaire
F	Female
FGF	Fibroblastic Growth Factor
Flk-1	Fetal Liver Kinase-1
Flt-1	FMS-like Tyrosine Kinase- 1
FMD	Flow Mediated Vasodilatation
FMS	Feline McDonough Sarcoma
H	Hispanic
HbA1c	Glycosylated Haemoglobin
Hct	Haematocrit
Hcy	Homocysteine
HDL-C	High Density Lipoprotein- Cholesterol
IC	Intermittent Claudication
ICAM	Intracellular Adhesion Molecule
IL-1β	Interleukin-1 Beta
IL-6	Interleukin-6
IMT	Intima Media Thickness
IQR	Inter-quartile Range
JVP	Jugular Venous Pressure
LDL-C	Low Density Lipoprotein- Cholesterol

Lp (a)	Lipoprotein A
M	Male
MCP	Monocyte Chemoattractant
MI	Myocardial Infarction
MRA	Magnetic Resonance Angiography
mRNA	Messenger Ribonucleic Acid
NA	Native American
NHW	Non-Hispanic Whites
NO	Nitric Oxide
NPV	Negative Predictive Value
PAD	Peripheral Arterial Disease
PAI	Plasminogen Activator Inhibitor
PB	Philip Bennett
PDGF	Platelet Derived Growth Factor
PlGF	Placental Growth Factor
PMA	Platelet Microaggregates
PMP	Platelet Microparticles
PP	Pulse Pressure
PPV	Positive Predictive Value
P-sel	P-selectin
PTA	Percutaneous Transluminal Angioplasty
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
s	Soluble

SAA	Serum Amyloid A
SBP	Systolic Blood Pressure
SD	Standard Deviation
SN	Sensitivity
SP	Specificity
sP-sel	Soluble P-Selectin
sTie2	Soluble Receptor Tyrosine Kinase 2
sTM	Soluble Thrombomodulin
TAT	Thrombin Antithrombin
TC	Total Cholesterol
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TG	Triglycerides
TGF-ß	Transforming Growth Factor- Beta
Tie-2	Receptor Tyrosine Kinase-2
TM	Thrombomodulin
TNF	Tumour Necrosis Factor
tPA	Tissue Plasminogen Activator
tRNA	Transfer Ribonucleic Acid
VCAM	Vascular Adhesion Molecule
VEGF	Vascular Endothelial Growth Factor
VWF	Von Willebrand Factor
WSS	Wall Shear Stress

SYNOPSIS

This thesis sets out to examine ethnic differences in the epidemiology and pathophysiology of peripheral arterial disease (PAD). Chapter 3 is a sub-study to the Ethnic-Echocardiographic Heart of England (E-ECHOES) community based screening study. Chapter 4 is a hospital based study.

Chapter 3.1 attempted to validate translated versions of the Edinburgh Claudication Questionnaire (ECQ) into languages of the Indian sub-continent (Hindi, Urdu, Bengali, Punjabi, Gujurati) and compare their sensitivity and specificity to the original English version used in the Edinburgh Artery Study. It also validated the questionnaire in 1st generation Black Caribbean UK migrants. This chapter's findings suggest that the ECQ is not as sensitive or specific a diagnostic tool in 1st generation Black Caribbean and South Asian UK migrants than in the indigenous White European population participating in the Edinburgh Artery Study, reflecting the findings of other diagnostic questionnaires in these minority ethnic groups.

Chapter 3.2 investigated ethnic differences in the prevalence of PAD in South Asians and Blacks and made associations with traditional cardiovascular risk factors. In this cross-sectional study this chapter found the prevalence of PAD was similar overall but South Asian females had significantly higher prevalence of disease, not explained but traditional cardiovascular risk factors.

Chapter 3.3 investigated ethnic differences in mean and maximum common carotid intima medial thickness (CCIMT) and made associations with traditional cardiovascular risk factors and PAD. This chapter found that Black ethnicity was related to greater mean and maximum CCIMT when compared to South Asians, even after adjusting for traditional cardiovascular risk factors. The presence of PAD independently predicted mean and maximum CCIMT adjusting for ethnicity, age and cardiovascular risk factors.

Chapter 3.4 investigated ethnic differences in circulating haemostatic and inflammatory biomarkers and their association with ankle brachial pressure index (ABPI) and mean and maximum CCIMT. This chapter found that amongst South Asians, Von Willebrand Factor (VWF) correlated with ABPI and mean and maximum CCIMT. In Blacks, D-dimer was higher in PAD than in normal ABPI participants. This chapter's findings may reflect different pathophysiological processes predominating in different ethnic groups.

Chapter 4.1 looked at ethnic differences in plasma expression of circulating markers of angiogenesis amongst South Asians, Blacks and Whites and also between healthy volunteers, risk factor controls and participants with traditional cardiovascular risk factors and cardiovascular disease. In healthy controls, angiogenin was higher in South Asians and Blacks compared to Whites. The differences were not influenced by the presence of other risk factors or the presence of stable cardiovascular disease.

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CHAPTER 1

INTRODUCTION

1.1 Background into Peripheral Arterial Disease

1.1.1 Introduction

Peripheral arterial disease (PAD) is an important healthcare problem in developed nations and is associated with considerable morbidity and mortality [Norgren et al. 2007]. PAD is the disease process resulting from stenosis of large peripheral arteries, exclusive of the coronary and intracranial cerebrovascular system, most commonly due to atherosclerosis. Most typically, it is referred to in relation to the lower limbs. PAD is an indicator of widespread atherosclerosis in other vascular territories, such as the cerebral and coronary circulations [Norgren et al. 2007]. There is also considerable overlap between PAD, cerebrovascular disease (CBVD) and coronary artery disease (CAD) [CAPRIE steering committee 1996; Pasternak et al. 2004], with the presence of PAD being associated with an increased risk of CBVD and CAD and their consequences [Golomb et al. 2006; Criqui et al. 1998, Dormandy et al. 1999]. People with PAD have 4-5 times greater risk of dying from a cardiovascular disease event compared to those without, and a 2-3 times greater all-cause mortality [Newman et al. 1993b; Criqui et al. 1992].

In patients with both asymptomatic and symptomatic PAD, repeated skeletal muscle ischaemia and subsequent reperfusion are associated with an increase in oxidative stress and

its subsequent production of reactive oxygen species (ROS) along with elevation of neutrophil counts and platelet activation. Whilst hypoxia is the main driver of angiogenesis, oxidative stress acts as a stimulus for angiogenesis and potentiates the atherogenic and thrombogenic profile seen in PAD.

Risk factors for the development of PAD are very similar to those of CAD and CBVD. Traditional risk factors include tobacco smoking, diabetes mellitus, dyslipidaemias, hypertension, advancing age and male gender [Makin et al. 2002; McDermott et al 2001; Hankey et al, 2006; Caro et al. 2005, Price et al. 1999; Norgren et al. 2007]. Diabetes mellitus and smoking are the strongest modifiable risk factors for PAD in predominantly White populations [American Diabetes Association 2003; Garcia 2006]. Age and sex are the most important non-modifiable risk factors in all ethnic groups [Dormandy et al. 1999]. Risk factors that reduce the risk of getting PAD include regular physical activity and moderate alcohol intake [Dieter et al. 2002].

In recent years, several new plausible risk factors for atherosclerosis have been proposed including homocysteine [Smith et al. 2004; Khawaja et al. 2007], C reactive protein (CRP) [Selvin et al. 2004; Khawaja et al. 2007], fibrinogen [Selvin et al 2004; Khawaja et al. 2007], lipoprotein (a) [Smith et al. 2004; Cheng et al. 1997; Tseng et al. 2004], increased platelet activity [Dieter et al. 2002] and hypercoagulability [Dieter et al. 2004]. The relationship between PAD and these cardiovascular risk factors have not been fully elucidated, although some may explain the ethnic variations in susceptibility to PAD [Khawaja et al. 2007].

1.1.2 Defining Peripheral Arterial Disease and its complications

The most widely used definition of PAD is a diminution in the ankle brachial pressure index (ABPI). The ABPI is calculated by dividing the systolic blood pressure at the ankle by the systolic blood pressure in the arm. The ABPI <0.9 has been found to be up to 95% sensitive [Dormandy et al. 2000; Hummel et al. 1978] and 95% specific [Hummel et al. 1978] in detecting angiogram positive disease, with little inter-observer variability after relatively little training [Matzke et al. 2003].

PAD can be classified clinically using either the Fontaine or Rutherford classifications [Figure 1.1] [Norgren et al. 2007]. While the majority of patients are asymptomatic, the most common clinical manifestation of PAD is intermittent claudication (IC) [McDermott et al. 2001], affecting a quarter of this population. Of the asymptomatic patients 5 to 10% develop symptoms of PAD over 5 years [Hooi et al. 1999]. Only a minority of patients with IC develop symptoms of critical leg ischaemia. The development of symptomatic disease is typically preceded by and dependent upon the development of multiple collateral blood vessel formation. It is thought that the extent of this collateral formation affects the clinical manifestation of PAD; thus insufficient angiogenesis, and a failure to develop collateral blood vessels, may be responsible for the different symptomatic presentations of IC and CLI [Findley 2008].

Figure 1.1: Fontaine [Fontaine et al. 1954] & Rutherford [Rutherford et al. 1997] Classification Systems for Peripheral Arterial Disease

Fontaine		**Rutherford**		
Stage	Clinical	Grade	Category	Clinical
I	Asymptomatic	0	0	Asymptomatic
IIa	Mild claudication	I	1	Mild claudication
IIb	Moderate-severe claudication	I	2	Moderate claudication
		I	3	Severe claudication
III	Ischaemic rest pain	II	4	Ischaemic rest pain
IV	Ulceration or gangrene	III	5	Minor tissue loss
		IV	6	Ulceration or gangrene

1.1.3 Epidemiology of Peripheral Arterial Disease

Several population based studies [Fowkes et al. 1991; Meijer et al. 1998; Diehm et al. 2004] based on predominantly White European populations have found the prevalence of PAD to be between 6 to 18% over the age of 55. The prevalence rises with age and has been found to be approximately 20% in people over 70 years of age [Regensteiner et al. 2002] and up to 60% in the over 85 age group [Meijer et al. 1998]. There has however, been very little research into the prevalence of PAD in non-White populations although previous population based studies have shown variations in the prevalence of this disease amongst different ethnic groups [Table 1.1] [Premalatha et al. 2000, Criqui et al. 2005; UK Prospective Diabetes Study Group 1994; Mohan et al. 1995, Chaturvedi et al. 2007; Collins et al. 2005; McDermott et al.2005].

Table 1.1 Populations based studies investigating Peripheral Arterial Disease

Study	Country	Race/Ethnic Group Studied	Sample Size	Age	PAD Prevalence (%)
Fowkes et al. 1991	UK	European	1592	55-74	18.3
Meijer et al. 1998	Netherlands	European	6450	>55	19.1
Fabsitz et al. 1999	US	Native American Indians	4549	45-74	5.3
Premalatha et al. 2000	South India	Indian	631	>20	3.2
Diehm et al. 2004	Germany	European	6821	≥65	18
Selvin et al. 2004	US	African American Non-Hispanic White Hispanic	2174	>40	4.3 (AA:7.9; NHW:4.4; H:3.0)
Collins et al. 2005	US	African American Non-Hispanic White Hispanic	403	>55	16.6 (AA:22.8; NHW:13.2;H:13.7)
McDermott et al. 2005	US	African American Non-Hispanic White Hispanic Chinese	6560	45-84	3.7 M(NHW:2.7; Ch:1.1;AA:7.1;H:3) F(NHW:3.5;Ch:2.2; AA:6.1;H:1.7)
Criqui et al. 2005	US	African American Non-Hispanic White Hispanic Asian	2343	29-91	4.4 (AA:7.8; NHW:4.9 H:1.8;As:1.4)
Allison et al. 2006	US	African American Non-Hispanic White Hispanic Chinese	6653	45-85	4.3 (AA:7.2; NHW:3.6; H:2.4; Ch: 2)
He et al. 2006	China	Chinese	2334	≥60	19.8
Al-Sheikh et al. 2007	Saudi Arabia	Arabic	471	≥45	11.7
Garofolo et al. 2007	Brazil	Japanese	1008	≥30	20.4

Sritara et al. 2007	Thailand	Thai	2305	52-73	5.2
Carbayo et al. 2007	Spain	European	784	≥40	10.5
Sigvant et al. 2007	Sweden	European	5080	60-90	18
Kumar et al. 2007	South Africa	Black African	542	>50	29.3

AA: African American; As: Asian; Ch: Chinese; F: Female; H: Hispanic; M: Male; NHW: Non-Hispanic White; PAD: peripheral arterial disease; UK: United Kingdom; US: United States of America.

1.2 Ethnic differences in Peripheral Arterial Disease

1.2.1 Ethnic Differences in Vascular Disease

Ethnic minority groups make up 7.9% of the general population of the UK [Gill et al. 2007]. The largest of these being Asian/Asian British (50.2%) and Black/Black British (24.8%) [National Statistics online]. The remainder of this thesis will focus on these 2 largest minority ethnic groups within the UK.

There are well recognised differences in vascular disease in different ethnic groups. South Asians (People originating from India, Pakistan and Bangladesh) living in the UK have a higher mortality for ischaemic heart disease (IHD) then Europeans [Balarajan 1991] and Blacks (Black Caribbean and Black African) have a higher CBVD mortality in both sexes [Balarajan 1991]. However studies both in the UK and India suggest PAD prevalence is lower in South Asians than in White Europeans in general population, diabetic and CAD cohorts [Premalatha et al. 2000; UK Prospective Diabetes Study 1994; Mohan et al. 1995; Chaturvedi et al. 2007]. Traditional risk factors could not account for the observed difference in PAD in a recent UK study [Chaturvedi et al. 2007] as Indians are exposed to a number of risk factors which should increase their risk of PAD, such as dyslipidaemia and diabetes [Bhopal et al. 1999].

Several US studies [Table 1.1] found the prevalence of PAD in African Americans (AA) to be higher than in Non-Hispanic Whites (NHW). Compared to NHW, AA also had a significantly worse risk factor profile, with the exception of dyslipidaemia [Allison et al.

2006]. However differences in prevalence were only partially attributable to higher rates of diabetes mellitus (DM) and hypertension in this ethnic group [Criqui et al. 2005]. In the National Health And Nutrition Examination Survey (NHANES), when adjusting for all traditional risk factors the odds ratio (OR) for PAD in AA over NHW was 1.67. This OR reduced when further adjusting for novel risk factors suggesting that novel risk factors play some role at least in their susceptibility to PAD [Allison et al. 2006].

Whilst the prevalence of risk factors is variable amongst different ethnic groups it is thought that the underlying pathophysiology of arterial disease is affected by ethnicity and race and that some ethnic variations in risk factors may be genetically based.

1.2.2 Ethnic Differences in Traditional Cardiovascular Risk Factors and their association with Peripheral Arterial Disease

1.2.2.1 Tobacco Smoking

Smoking tobacco is a major risk factor in the development of PAD [McDermott et al. 2001; Caro et al. 2005] as a sequel of atherosclerosis [Fowkes et al.1992]. It can result in a 7 fold increase in PAD [Heliovaara et al. 1978] and its impact persists with advanced age [Kannel et al. 1994]. Smoking has been related to elevated CRP, fibrinogen and homocysteine, novel risk factors, in both white and non-white ethnic groups [Bazzano et al. 2003].

The Edinburgh Artery Study reported a dose dependent relationship between smoking and PAD [Price et al.1999]; a finding supported by other, more recent studies [Willigendael et al. 2004; Fowler et al. 2002]. NHANES found PAD prevalence increased sequentially from never smokers to ex-smokers to current smokers across all ethnic groups [Allison et al.2006]. In patients with PAD, smoking is associated with increased progression of disease and increased risk of amputation [Lassila et al. 1988]. It also reduces the effectiveness of antiplatelet medication in such patients [Lepantalo et al. 1991]. Cessation is associated with reduced disease progression and increased survival [Jonason et al. 1987].

The Prevalence of smoking varies among ethnic groups and gender. Even within the South Asian group there is considerable variation; for example Bangladeshi and Pakistani men having the highest and Indians the lowest of the male smokers and low rates of smoking amongst all of the female South Asians [Bhopal et al. 1999]. In the US, Asian Indians were much less likely to be current smokers than NHW [Mohanty et al. 2005].

There is limited evidence investigating smoking and PAD in ethnic groups. A population based study in South India [Premalatha et al. 2000] found that smokers had a 2.7 times higher risk for PAD, although the result did not reach statistical significance. A recent UK study on Indian Asians and Europeans with equivalent rates of CAD found the prevalence of PAD to be much lower in the former group [Chaturvedi et al. 2007], with smoking rates also lower in Indians. Adjustment for pack years reduced the significance of ethnicity considerably but could not account for the ethnic differences in PAD observed [Chaturvedi et al. 2007].

South Asian smokers were found to have a four times greater prevalence of CAD than non-smokers in a previous cross-sectional study [Bhopal et al. 1999], a similar elevation in risk to Europeans. However as a cross-sectional study causal associations between risk factors and PAD cannot be made and results should be interpreted with caution. Indeed only 1 to 5% of Asian females smoke yet they have double the risk of suffering CAD than White European females [Bhopal et al. 1999]. It could be perceived that with the overlap that exists with chronic vascular disease at different sites in predominantly White populations, there would be a similar relationship in ethnic minority groups. Whereas South Asians are at increased

risk of premature death from CAD [Chaturvedi et al. 2003; Lane et al. 2005] as compared to White European populations, limited data suggests that the prevalence of PAD in this group is much lower than in Europeans [Premalatha et al. 2000]. Another hypothesis suggests that either South Asians are not living long enough to develop symptomatic PAD or other - as yet undetermined - factors are responsible for the development of atherosclerosis in different vascular territories.

Evidence investigating the relationship between Black ethnicity, smoking and PAD is conflicting and comes only from cross-sectional studies. The United Kingdom Prospective Diabetes Study (UKPDS) [UKPDS 1994] found that PAD was less frequent in Blacks than in White Europeans and that the proportion of never smokers was greater in the former group. AAs on the other hand, were found to have a higher prevalence of PAD compared to NHW yet had a lower prevalence of smoking in another study [Collins et al. 2003] yet in another [Kuller et al. 1998] the percentage of current smokers was higher in AA with sub clinical vascular disease than in NHW. With methodological differences between these studies it is difficult to elucidate whether smoking confers a greater risk for PAD in Blacks than in Whites; especially as these studies are all cross-sectional studies and as such one must be very cautious when interpreting the data as causal associations shouldn't be made in such studies between socio-demographic factors and PAD.

1.2.2.2 Diabetes mellitus and impaired glucose tolerance (IGT)

Diabetes mellitus (DM) is a major cardiovascular risk factor, and the incidence of type 2 diabetes is rising in developed nations as a consequence of lifestyle changes resulting in increased obesity.

DM is associated with an increased risk of stroke and with a two- to four-fold increased risk of developing CAD [Luscher et al. 2003] and PAD [Newman et al.1993a]. DM and smoking are together the most important risk factors for the development and progression of PAD [Smith et al. 2004]. In diabetic patients, the risk of PAD is increased by age, duration of DM, blood glucose control and peripheral neuropathy. Approximately 5 to 10% of PAD patients have type 1 and 90 to 95% have type 2 DM [Creager et al. 2003]. Blood glucose control seems to be an important factor for PAD [Selvin et al. 2006] and it has been estimated that with each 1% increase in glycosylated haemoglobin (HbA1c) level comes a 28% increased risk of incident PAD [UKPDS 1998]. In the Framingham Heart Study, 20% of symptomatic PAD patients had DM [Murabito et al. 1997]. Other studies have found the prevalence of PAD in diabetics to be 20% in people over 40 years old [Elhadd et al. 1999] and 29% in people aged over 50 [Hirsch et al 2001]. IC and Critical leg ischaemia are also more common in patients with DM [American Diabetes Association 2003]; the risk of IC being two-fold higher [Norgren et al. 2007].

The prevalence of type 2 DM in the UK is much higher in African Caribbean and South Asian groups compared to the general population [Health Survey for England 2004]. Various

studies have shown that 16 to 17% African Caribbeans and 20% of South Asians aged between 40 and 64 years have type 2 DM compared to 3 to 5% of Europeans [Chaturvedi et al. 1994; Diabetes in the UK 2004]. After standardising for age, South Asians have a 3 to 6 fold and African Caribbeans a 2.5-fold greater risk of DM than the general population [HSE 2004]. By contrast, the prevalence of DM in rural India is 2 to 3% and approximately 8% in urban areas [Ramachandran et al. 1992].

Interestingly, a migration study found fasting glucose to be much higher in South Asians living in West London, UK than their siblings living in the state of Punjab, India [Bhatnagar et al. 1995]. This difference infers that there could be a genetic susceptibility to the development of DM, which is exacerbated by lifestyle differences and environmental factors which arise from living in developed countries. However, this was not supported by another migration study comparing Gujuratis living in Birmingham, UK and age, gender and caste-matched non-migrant Gujuratis remaining in their villages of origin in Navsari, India, which found the diabetes prevalence to be similarly high in both native Indians and UK migrants [Patel et al. 2006]. Both studies were matched on age and gender and compared migrants living in urban areas of the UK with rural areas in India. Both studies found UK migrants to have higher BMI, higher systolic blood pressure and higher fasting cholesterol. Whilst Patel et al. 2006 found no difference in impaired glucose tolerance between migrants and those in rural India, they reported that diabetes and impaired glucose tolerance were less frequently associated with excess metabolic cardiovascular risk factors in India [Patel et al. 2006].

A cross-sectional study, in subjects with a mean age of 46 years, in South India reported a prevalence of PAD of 6.3% amongst diabetics compared to 3.2% in the whole population

[Premalatha et al. 2000] This contrasts with a cohort study from the US, with subjects between 50 and 70 years of age, which reported the PAD prevalence to be 22% in its diabetic cohort as compared to 3% in people with normal glucose tolerance [Beach et al. 1998]. This discrepancy in PAD prevalence in diabetics may be due to differences in the age of subjects between the 2 studies.

When compared to diabetic Whites in other studies, the prevalence of PAD in diabetic South Asians was found to be much lower [Abbot et al. 2005; Chaturvedi et al 2001]. Chaturvedi et al. 2007 found Indian Asians to have a lower prevalence of PAD than White Europeans with equivalent CAD levels despite Indians having a higher prevalence of insulin resistance and a higher HbA1c [Chaturvedi et al 2007].

AAs with diabetes were found to have a higher prevalence of PAD than NHW in the US [American Diabetes Association 2003] but no difference in prevalence between Blacks and Whites were apparent in a UK study [Abbot et al. 2005]. Interestingly amongst patients with PAD in the US a similar prevalence of DM was found between AA and NHW [Collins et al. 2003]. As with smoking, these studies have all been cross-sectional in design and as such it is difficult to establish a link between diabetes and PAD which accounts for the apparent increase in prevalence of this disease in Blacks.

1.2.2.3 Dyslipidaemia

Abnormalities in the components of the lipid profile are associated with PAD. These do not appear to carry the same importance as smoking and DM in its development [Faxon et al. 2004]. The ratio of Total Cholesterol (TC): High Density Lipoprotein Cholesterol (HDL-C) is the strongest lipid predictor of PAD risk [Norgren et al. 2007; Ridker et al. 2001]. The most frequent dyslipidaemia associated with PAD is elevated triglycerides and low HDL-C [Smith et al. 2004]. The association between Low Density Lipoprotein Cholesterol (LDL-C) and PAD appears to be weaker [Pasternak et al. 2004] than for HDL-C and triglycerides (TG), and furthermore weaker than its association with the development of CAD.

In the UK no statistically significant differences in TC were found between ethnic groups, with the exception of African Caribbean females [Health Survey for England 1999]. In the US, the TC: HDL-C ratio has also been found to be quite similar in NHW, African Americans and Asians [Criqui et al.2005]. South Asians appear to have a less favourable lipid profile than other ethnic groups with a lower HDL-C and higher TG than White [Chaturvedi et al. 1994; HSE 1999; Miller et al. 1984; Li et al. 2006; Zoratti et al. 1998]. This finding is apparent even in children [Whincup et al. 2002]. These abnormalities in HDL-C and TG become more pronounced on migration from the Indian subcontinent [Bhatnagar et al. 1995; Patel et al. 2006], suggesting both environmental and genetic factors are at play. On the other hand Blacks have a more favourable lipid profile than White [Allison et al. 2006; Whincup et al. 2002; Lip et al 2007] with higher HDL-C levels [Kuller et al. 1998; HSE 1999]. Interestingly statistically significant disease/race interactions for

LDL-C levels have been found, showing a stronger association in Blacks (especially in women) [Kuller et al. 1998].

There are limited data linking PAD to dyslipidaemia in ethnic groups. A population based study in India linked hypercholesterolaemia and LDL-C to the development of PAD (ORs 1.4 and 1.5 respectively) [Premalatha et al. 2000], although the findings were not statistically significant. A South African study found Blacks with PAD had lower TC, TG and HDL levels than White with PAD [Keenen et al. 1985]. The discordance between lipid profile and PAD risk in South Asians and Blacks suggests that dyslipidaemia does not play as important a role in its development as in the pathogenesis of CAD.

1.2.2.4 Hypertension

Hypertension is a major risk factor for all vascular disorders and is associated with a 2 to 3 fold risk for PAD [Norgren et al. 2007]. Several components of blood pressure, including pulse pressure (PP), systolic blood pressure (SBP) and diastolic blood pressure (DBP) have been shown to be independent cardiovascular risk factors [Safar et al. 2003]. SBP in particular is a major risk factor for CAD and stroke. In PAD hypertension is a risk factor for both symptomatic and asymptomatic disease [Dieter et al. 2002; Murabito et al. 1997] and the degree of hypertension is also closely linked to the development of PAD [Murabito et al. 1997].

PAD and hypertension are associated diseases with 35-55% of patients with PAD also having hypertension at presentation [Cheng et al. 1999; Vidi et al. 1996; Makin et al. 2001]. The prevalence of PAD in patients with DM increases with the presence of systolic hypertension in all racial groups. If hypertension in these patients is controlled, the progression of PAD can be slowed [Palumbo et al.1991].

The prevalence of hypertension has previously been reported as being higher in Blacks compared to Whites in the UK [Agyemang et al. 2003]. Black people have been found to develop hypertension at an earlier age [Brown 2006], have more severe disease [Cooper et al. 1997], have worse BP control, have a different distribution of target organ damage [Brown 2006] and a raised mortality from hypertension; perhaps 1.5 times higher than national average [Agyemang et al. 2003]. Interestingly, when comparing the prevalence of hypertension internationally between White European populations and Blacks, a wide

variation was found in hypertension prevalence [Cooper et al. 2005]. In fact Black ethnicity did not have unusually higher prevalence of hypertension than Whites [Cooper et al. 2005]. In some studies, the prevalence of hypertension in the South Asian immigrant population in the UK is also significantly higher [Cappuccio et al. 1997; Primatesta et al. 2000]. However, the mean population blood pressure in these ethnic groups is similar to that of White European populations [HSE 2004], possibly due to heterogeneity of the South Asian and White populations.

A meta-analysis of hypertension in South Asians in UK found that they had lower mean systolic but higher diastolic blood pressures in both males and females compared to whites [Agyemang et al. 2002]. The prevalence of hypertension was higher in South Asian males but results were inconclusive in females [Agyemang et al. 2002]. The researchers commented that mean BP and prevalence were different amongst South Asian subgroups but they were usually combined into one homogenous group [Agyemang et al.2002]. This is an important finding given that the classic risk factor profile of these subgroups differs, which may affect any analyses associating them with PAD. A meta-analysis in India found that hypertension is rising in general, and significantly more so in urban than rural populations [Gupta et al. 1996], suggesting that changes in lifestyle and environmental factors play an important role. This is supported by a recent UK migrant study, which found a higher prevalence of hypertension in migrants than in indigenous Indians [Patel et al. 2006].

While being a risk factor for PAD, hypertension appears to have a stronger association with CAD and both haemorrhagic and ischaemic stroke. The association appears to be greater for ischaemic stroke [Perry et al. 2000]. Treating hypertension reduces the incidence of both haemorrhagic and ischaemic stroke [Perry et al. 2000]. This finding may explain why

cerebrovascular disease is more common in Blacks [Dieplinger et al. 1999; Raleigh et al. 1996]. It may also explain the higher risk of and mortality from stroke in South Asians compared to White in the UK [Wild et al. 1997].

There is a paucity of data regarding the association between hypertension and PAD in different ethnic groups. US Studies have shown that among patients with PAD, the prevalence of hypertension was higher in AA than NHW [Criqui et al.2005; Collins et al. 2003] and lower in Asians [Criqui et al. 2005]. A population based study in India found that PAD patients had a significantly higher mean SBP than those without PAD [Premalatha et al. 2000] and the OR for hypertension and PAD was 2.7 [Premalatha et al. 2000]. However, more research needs to be undertaken to explain the variation in PAD with hypertension in different ethnic minority groups.

1.2.3 The metabolic syndrome

Many studies have identified a pattern of inter-related metabolic disturbances that are associated with cardiovascular risk, which are referred to as the so-called 'metabolic syndrome'. The current definition of metabolic syndrome requires the presence of 3 or more of the following: Increased waist circumference, Reduced HDL cholesterol, Increased Triglycerides, Hypertension, Impaired fasting glucose or DM [International Diabetes Federation 2008]. Metabolic syndrome varies by ethnic group and is especially prevalent amongst South Asians [Ridker et al. 2001]. Even though African Caribbeans have a tendency for insulin resistance and an increased risk of DM, they appear to have a more favourable lipid profile than South Asian [Lip et al. 2007] and thus do not fit the typical definition of metabolic syndrome [Whitty et al. 1999].

Metabolic syndrome has been associated with the development and progression of and more advanced PAD [Wild et al. 2006; Olijhoek et al. 2004; Lahoz et al. 2006]. However, with the components of metabolic syndrome being associated with low ABPI, defining the syndrome does not add any more per se to the patient's classical risk factor status when assessing the risk of suffering from asymptomatic PAD [Lahoz et al. 2006]. While this syndrome has been associated with PAD, it seems clear that ethnicity/ race play a greater role in conferring susceptibility to this disease.

1.2.4 Ethnic Differences in Peripheral Arterial Disease Distribution

As well as the variations that exist in vascular risk factors, the anatomical distribution of vascular disease appears to differ between ethnic groups. Black people have an increased incidence of cerebrovascular disease and end stage renal disease than White and a lower incidence of CAD, whereas South Asians appear to have a higher incidence of CAD [Chaturvedi et al. 2003]. Whites, on the other hand, appear to have a higher rate of abdominal aortic aneurysm (AAA) [Kent et al. 2010; Salem et al. 2009; Hobbs et al. 2003]. Interestingly when AAAs are found in Blacks, they present at a significantly younger age than White and atherosclerosis is less likely to be the causative factor [Robbs et al. 1985; Dardik et al. 1999]. In people with strokes, Black people appear to have more cerebral small vessel and less large vessel atherosclerotic disease than White [Markus et al. 2007]. They also have a greater mortality from this disease than White [Balarajan et al. 1991].

Previous studies have indicated that African Caribbeans and South Asians seemed to report more distal PAD than the general population [Collins et al. 2005; Lip et al. 2007; Hobbs et al. 2003; Robbs et al. 1985; Deneuville et al. 2008]. Examples of these studies are illustrated in table 1.2. In Indians, *thromboangiitis obliterans* appears to contribute to this increase [Shead et al. 1978]. In a previous hospital based study in India, this disease reflects the younger age of presentation of patients with distal PAD [Shead et al. 1978]. The study by Chaturvedi et al. found that Indian Asians had less lower limb atherosclerosis than Europeans, while common carotid intima-medial thickness (CIMT) did not differ [Chaturvedi 2007] and disease distribution was similar. This finding is consistent with other

studies reporting lower rates if limb amputation in Indian Asians compared to White Europeans [Premalatha et al. 2000; Mohan et al. 1995; Chaturvedi et al. 2001].

It is not known whether distal PAD is due to genetic differences in disease distribution or the fact that the incidence of DM is higher in South Asians and Blacks than in White and DM has been shown to be associated with distal PAD. There is evidence to suggest that there are regional differences in atherosclerosis risk susceptibility to specific risk factors [Chaturvedi et al. 2007]. DM usually affects smaller arteries and therefore is associated with femoro-popliteal and tibial PAD [American Diabetes Association 2003; Jude et al. 2001]. This pattern differs from other risk factors such as smoking and hypertension which are associated with more proximal disease in the aorto-iliac vessels [American Diabetes Association 2003]. However, the finding of distal PAD in South Asians and Blacks is apparent even after adjusting for the increased prevalence of DM [Leggeter et al. 2002].

Table 1.2 Examples of studies demonstrating ethnic differences in disease distribution of peripheral arterial disease

Study	Country	Race/ Ethnic Group Studied	Sample Size and Sex	Age (years)	Summary of Findings
Kent et al. 2010	US	NHW, Hispanic, AA, Asian, Native American	3.1 million male & female	>50	Prevalence of AAA: NHW:0.8%, H:0.28%, AA:0.39%, As:0.29%, NA: 0.95%
Salem et al. 2009	UK	White Asian	19014 male	>65	Prevalence of AAA: White: 4.69%, As: 0.45%
Wilson et al. 2008	US	NHW, AA	606000 male	>65	Prevalence of AAA: NHW: 1.41%, AA: 0.55%
Deneuville et al. 2008	West Indies	African Caribbean	754 male & female	Mean age 73+/-10	Vascular disease distribution: Infra-genicular: 86%, Femoro-popliteal: 51% Aorto-iliac: 7%
Robbs et al. 1985	South Africa	White, Black, Indian	1342 male & female	Mean age White: 59 Black: 54.8 Indian: 54.6	Vascular disease distribution: White: Extra-cranial cerebrovascular 10.1%, Aorto-iliac: 30.5% Femoro-popliteal: 22%, Tibio-peroneal: 9.4%, AAA: 25%, Indian: Extra-cranial cerebrovascular: 12.7%, Aorto-iliac: 33.3%, Femoro-popliteal: 33% Tibio-peroneal: 14.6% AAA: 3.9%, Black: Extra-cranial cerebrovascular: 2.7%, Aorto-iliac: 34.1% Femoro-popliteal: 36.8% Tibio-peroneal: 10.2% AAA: 5.6%

AA: African American; AAA: Abdominal aortic aneurysm; As: Asian; H: Hispanic; NA: Native American; NHW: Non-Hispanic White

1.2.5 Summary of Ethnicity and PAD

PAD is an important healthcare problem worldwide due to morbidity and mortality associated with this condition. Most population based studies investigating PAD prevalence and risk factors for its development and progression have been based on predominantly White European groups. Much less is known about the characteristics of this disease in minority ethnic groups. As a consequence, decisions regarding management of PAD, including risk factor modification, have been made on the basis of majority White European populations. Understanding the epidemiology of PAD amongst ethnic groups is relevant, given that the proportion of minority ethnic groups in the UK rose by 53% between 1991 and 2001 [National Statistics Online 2008] and it is likely to rise further in the next census of 2011. It is also evident that there is considerable heterogeneity in risk factors within the South Asian ethnic group, which may affect any conclusions made linking PAD and ethnicity to traditional vascular risk factors.

Although South Asians have an apparently worse risk factor profile than the general population and a greater risk of CAD, they appear to have a lower prevalence of PAD. This could be a reflection of the higher premature death rate from CAD in South Asians compared to the general population [Balarajan et al. 1991]. Susceptible South Asians may not live long enough to develop symptoms of PAD. Given that PAD prevalence risk increases with age the apparent finding of lower prevalence in South Asians may be genuine; however, communication difficulties in describing symptoms of PAD which may result in its under diagnosis in this group. Amongst people of Black ethnicity, there is a discrepancy as to whether or not they have a higher prevalence of PAD.

Given the lack of concordance between risk of CAD, cerebrovascular disease and PAD in South Asian and Black ethnic groups it would appear likely that the development and distribution of chronic vascular diseases has some genetic basis. With little data it is hard to make firm conclusions regarding the importance of risk factors in the development of PAD in different minority ethnic groups

1.3 Peripheral Arterial Disease and Virchow's Triad

1.3.1 Introduction

Over 150 years ago, Virchow described a triad of abnormalities (abnormal blood flow, abnormal vessel wall and abnormal blood constituents) associated with thrombus formation (thrombogenesis). Given that the underlying pathophysiological mechanisms involved in the development, progression and complications of PAD are atherogenesis and thrombogenesis, abnormalities in Virchow's triad, initially used with reference to venous thrombosis [Virchow et al. 1856], has been discussed in relation to this disease [Makin et al. 2002; Chung et al. 2003/2004; Lowe et al. 2003/2004]. Indeed there has been increasing interest in the components of Virchow's triad in understanding the development and progression of PAD, as well as its symptomatic complications.

1.3.2 Defining Virchow's triad

The original components of the triad have been updated for the 21st century- for example, *abnormal vessel wall* now relates to endothelial damage/ dysfunction and structural changes within the vessel wall, whilst *abnormal blood constituents* refers to abnormalities in platelets, as well as coagulation and fibrinolytic pathways; and *abnormal flow* refers to abnormalities in haemorrheology and turbulence at bifurcations and stenotic regions [Lip et al. 2004]. Improvements in biochemical techniques, notably doppler flow analysis, enzyme-linked immunosorbant assay (ELISA) and coagulation bioassays, have allowed components of Virchow's triad to be quantified and studied in relation to their role in arterial thrombosis.

It should not be forgotten that traditional cardiovascular risk factors, including smoking, hypertension, hyperlipoproteinaemia and hyperglycaemia, play an important role in initiating and accelerating atherogenesis [Figure 1.2], also by altering components of Virchow's triad. Studies have reported that abnormalities in markers of thrombogenesis appear to be related to – and even additive to - conventional risk factors. For example, subjects in the highest tertiles of both fibrinogen *and* total or low-density lipoprotein (LDL) cholesterol are at greatest cardiovascular risk compared to subjects with high cholesterol but modest fibrinogen [Ridker1997]. There is also mounting evidence of the prognostic value of various markers of thrombogenesis, which suggests that they are not merely consequences of atherothrombotic diseases, but may actively contribute to the pathogenesis of vascular disease and its complications [Lip et al. 2004].

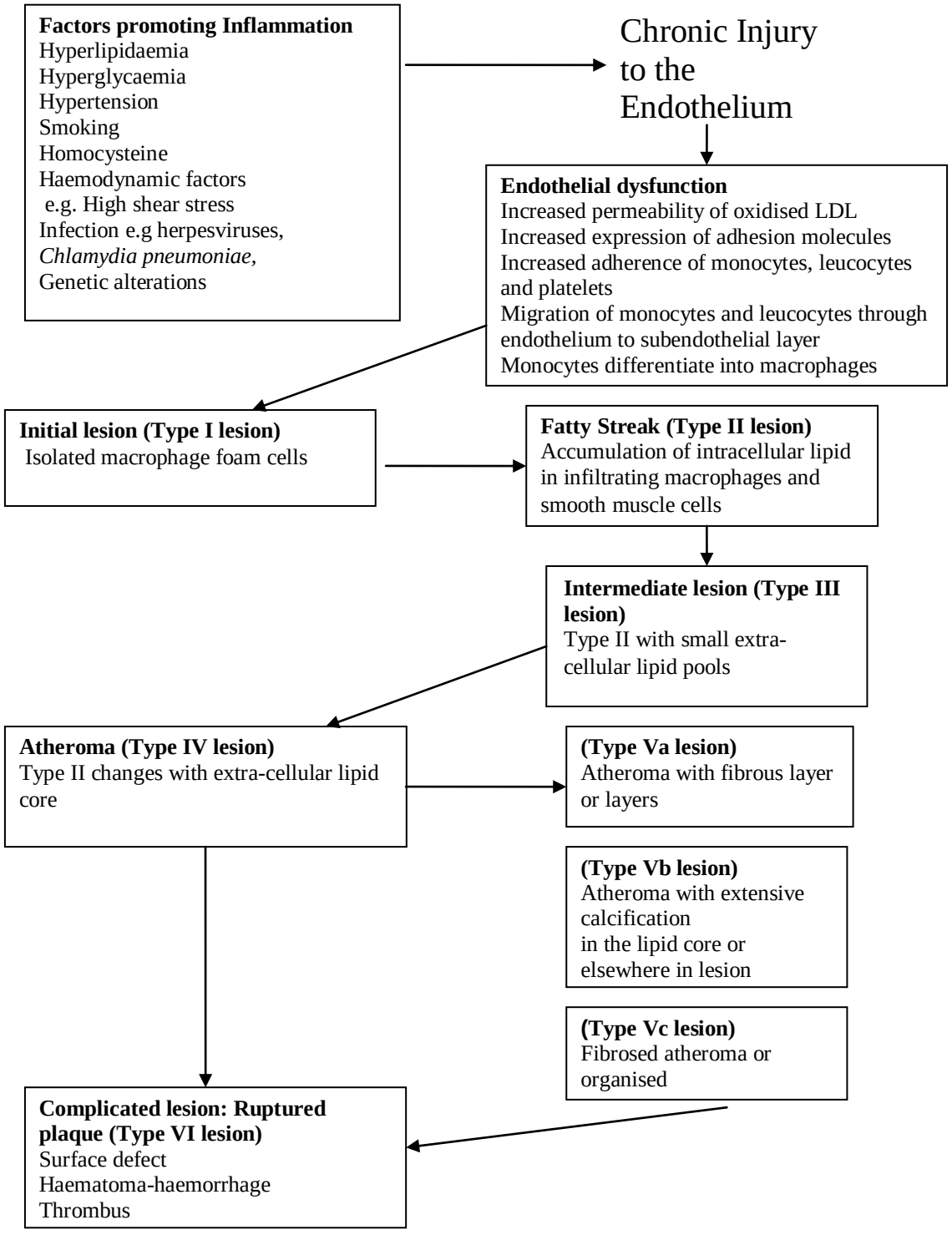


Figure 1.2: Stages of atherogenesis (based on Ross et al. 1999 and American Heart Association Classification of Atherosclerotic lesions [Stary et al. 1995])

1.3.3 "*Abnormal Vessel Wall*" and Peripheral Arterial Disease

Endothelial dysfunction is the earliest pathological process in atherogenesis [Ross 1999] [Figure 1.2]. Atheroma, typically occurring at branches, bifurcations and curvatures, disrupts normal laminar blood flow, leading to turbulence and altered wall shear stress (WSS). The latter causes platelet activation and aggregation, which enhances thrombus formation at these sites. Rupture of an atherosclerotic plaque, leads to exposure of its thrombogenic lipid core, which precipitates thrombus formation, causing occlusion with resulting ischaemia [Hiatt et al. 2002].

With endothelial damage or dysfunction there is a reduction in the bioavailability of vasodilators, such as nitric oxide (NO) leading to decreased endothelium-dependent vasodilatation and disruption of normal vascular homeostasis. This is characterised by a state of endothelial activation, which predisposes the endothelial micro-environment to a pro-inflammatory, hypercoagulable or pro-thrombotic state, which promotes all stages of atherogenesis. Many of the traditional risk factors are associated with the over-production of reactive oxygen species (ROS) or increased oxidative stress [Cai et al. 2000]. By reacting with NO, ROS may decrease the bioavailability of NO, impairing flow-mediated vasodilatation (FMD). Whilst PAD has previously been associated with a reduction in FMD [Brevetti et al. 2003] protocols for assessing FMD still vary among different laboratories and can be operator dependent [Anderson 1999; Coretti et al. 2002]. This decreases the feasibility of this measurement technique as a screening tool for endothelial dysfunction in large-scale multinational epidemiological studies [Bonetti et al. 2003].

The *abnormal blood vessel wall* in PAD can also be demonstrated by abnormalities of levels of specific plasma markers of endothelial damage/dysfunction as well as by abnormalities in vessel structure, for example, intima- medial thickness and arterial stiffness, as described below. Examples of studies reporting positive associations between markers of endothelial dysfunction and PAD are shown in Table 1.3.

Table 1.3: Examples of studies reporting positive associations between components of Virchow's Triad and peripheral arterial disease

Components of Virchow's Triad:
A: *Abnormal blood vessel wall*
B: *Abnormal blood constituents*
C: *Abnormal blood flow*

ABPI: ankle brachial pressure index; PAD: peripheral arterial disease; IC: intermittent claudication; CLI: critical limb ischaemia; MI: myocardial infarction; TIA: transient ischaemic attack; CAD: coronary artery disease; AAA: abdominal aortic aneurysm; BMI: body mass index; PTA: percutaneous transluminal angioplasty; TF: tissue factor; TFPI: tissue factor pathway inhibitor; CRP: C-reactive protein; IL: interleukin; VCAM-1: vascular adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1; Hcy: homocysteine; PAI-1: plasminogen activator inhibitor-1; tPA: tissue plasminogen activator; TM: thrombomodulin; SAA: serum amyloid A; IMT: intima medial thickness; AIx: aortic index; P-sel: p-selectin; VWF: von Willebrand factor; Lp(a): lipoprotein (a); TAT: thrombin antithrombin three; PMA; platelet microaggregates, MCP-1: monocyte chemoattractant protein-1; PMP: platelet microparticles; WSS: wall shear stress, CEC: circulating endothelial cells; Hct: haematocrit.

Study & Population	**Markers Studied**	**Summary of significant (P<0.5) associations**	**Components of Virchow's Triad**
Khaleghi et al. 2009 1051 African Americans, 894 Non-Hispanic Whites	Fibrinogen, D-dimer, Factors V, VII, VIII, VWF, Antithrombin III	Elevated fibrinogen and D-dimer were the only markers independently associated with lower ABPI in African Americans and Non-Hispanic Whites	A, B
Bartlett et al. 2009 785 men with PAD	Fibrinogen	Baseline fibrinogen was an independent predictor of 3 and 6 month risk of death adjusting for baseline risk factors including age, total and HDL cholesterol, triglycerides, SBP, DBP, smoking, previous MI, stroke, stable angina and use of antiplatelets. Individual led predictions may not be improved materially by measurement of fibrinogen.	B
Gosk-Bierska et al. 2008 62 patients with IC, 20 controls	TF, TFPI	Patients with PAD had higher TF, total TFPI and truncated TFPI than controls. Full-length TFPI was lower in patients with PAD than in controls	A
McDermott et al. 2008 423 patients with	D-dimer, CRP, IL-6, VCAM-1, ICAM-1, Hcy	Elevated levels of all of these factors were associated with poorer 6 minute walking performance. Increased D-dimer,	A, B

PAD		IL-6, VCAM-1 and Hcy were associated with slower usual paced 4 metre walking speed	
Mota et al. 2008 36 patients with PAD, 30 Controls	D-dimer, plasminogen, prothrombin fragment 1 & 2, PAI-1, TM	Patients had higher levels than controls. Inverse correlations between plasminogen and PAI-1	A, B
Heneghan et al. 2008 225 patients with CLI undergoing revascularisation procedures	Hcy	Patients with raised Hcy had significantly lower primary, assisted primary and secondary patency rates at all intervals to 36 months. Mean amputation survival was lower in patients with raised Hcy	B
Hogh et al. 2008 452 patients with symptomatic PAD	CRP	CRP levels were higher overall among patients developing primary endpoints (death, lower limb amputation, peripheral revascularisation) or secondary endpoint (thrombosis of lower limbs, MI, stroke, TIA)	B
Vidula et al. 2008 377 patients with PAD	D-dimer, CRP, SAA	Higher levels of these factors was associated with higher all cause and higher cardiovascular mortality among patients who died within 1st 2 years of measurement in people with PAD	B
Sodhi et al. 2007 195 patients >40 years free from cardiovascular disease and symptomatic PAD	IMT	There was a significant correlation between ABPI and common carotid IMT	A
Khaleghi et al. 2007 475 Patients with PAD	AIx.	AIx. Was independently associated with lower ABPI; association modified by age	A
Brewer et al. 2007 106 Patients with PAD	AIx.	High AIx associated with lower walking distance in people with PAD	A
Rajagapolan et al. 2007 182 patients with	P-Sel Fibrinogen	Patients with sub-critical limb ischaemia had significantly enhanced ADP stimulation, P-Sel expression and bound fibrinogen.	B

symptomatic PAD		Also had higher TRAP platelet aggregation	
Cleanthis et al. 2007 19 patients with IC and 10 patinets with CLI taking aspirin 12 controls not on aspirin	PMA, P-Sel, MCP-1	P-Sel, PMA and MCP-1 were all higher in CLI compared to IC and controls despite aspirin	B
Tzoulaki et al. 2007 1519 men & women	CRP, fibrinogen, Lp(a), Hct, IL-6, ICAM-1, VCAM-1, blood viscosity. plasma viscosity, tPA	CRP, fibrinogen, Lp(a) and Hct were significantly associated with PAD after 17 years follow up. Significant trend between higher levels of ICAM-1, D-dimer, tPA and Hct and worsening disease from no disease to moderate (IC) and severe (CLI or surgical intervention). IL-6, ICAM-1, Lp(a), fibrinogen, tPA and D-dimer and al rheological markers were significantly elevated at baseline in patients who experienced symptomatic PAD during 17 year follow up.	A, B, C
Iwashima et al. 2006 40 patients with PAD 48 controls	ICAM-1, VCAM-1, Adiponectin, CRP	Adiponectin was lower in PAD than without. Adiponectin, sICAM-1, sVCAM-1, CRP were independently associated with ABPI	A
Allison et al. 2006 6814 men and women free from clinically apparent cardiovascular disease from AA, NHW, Hispanic and Chinese ethnic groups	IL-6, fibrinogen, D-dimer, Hcy, VWF	IL-6, fibrinogen, D-dimer and Hcy were all significantly associated with PAD after adjustment for traditional cardiovascular risk factors in this large cross-sectional study. These novel risk factors do not entirely explain the difference in ethnic-specific odds for PAD.	A, B
Kals et al. 2006 38 Patients with PAD 28 Controls	Arterial elasticity, oxidative stress	Patients with PAD had decreased large and small artery elasticity. Decreased arterial elasticity and high grade oxidative stress was found in patients with	A, C

		atherosclerosis.	
Spring et al. 2006 31 Patients with PAD 31 patients with AAA 37 Controls	WSS, erythrocyte aggregation, leucocyte count, fibrinogen, plasma viscosity, IMT	WSS was lower in patients with PAD and AAA than in controls. WSS was inversely related to erythrocyte aggregation, fibrinogen, leucocyte count, plasma viscosity and IMT.	A, B, C
Brevetti et al. 2006 154 patients with PAD	CRP, metabolic syndrome	Metabolic syndrome was present in 51.9% of PAD patients. Patients with an ABPI <0.64 were more likely to have metabolic syndrome than those with less severe PAD. PAD patients with metabolic syndrome had higher BMI & CRP than those without.	B
Unlu et al. 2006 45 patients with PAD 44 controls	D-dimer, CRP, fibrinogen, SAA	D-dimer, CRP, SAA were higher in PAD group than control group. PAD associated with moderately higher fibrinogen. CRP & serum amyloid A had an inverse relationship with ABPI. D-dimer & fibrinogen were related to lower ABPI	B
Kudoh et al. 2006 42 patients with CAD and PAD 56 CAD only 32 controls	Platelet aggregation	The level of small platelet aggregates was increased significantly in the PAD group compared with both CAD and control groups. Platelet aggregability was increased in patients with PAD with the degree of platelet aggregation being closely related to ABPI	B
Laxdal et al. 2006 139 vascular interventions on 103 patients with common iliac occlusive disease	Hcy, fibrinogen, d-dimer, activated protein C resistance	There was a significant association between patency rate and levels of fibrinogen, Hcy. The highest values of Hcy and fibrinogen were independent predictors of failure	B
McDermott et al. 2005 6570 men and women free from clinically evident disease	CIMT	Men and women with definite PAD, borderline ABPI or low-normal ABPI had significantly higher internal CIMT than those with normal ABPI. In men the association between ABPI and internal CIMT were significantly less inverse in White than in	A

		African Americans	
Cassar et al. 2005 132 patients with IC 30 CLI 40 controls	CRP, VWF, D-dimer, TAT	CRP, vWF, D-dimer and TAT were significantly raised in patients with IC compared to controls. Patients with CLI had significantly higher levels of CRP, VWF, and TAT than claudicants	A, B
Sofi et al. 2005 280 patients with symptomatic PAD 280 controls	PAI-1, Lp(a), Hcy, factor V leiden mutation, prothrombin variant	There was an association between PAD symptoms and prothrombin variant, altered levels of homocysteine, Lp(a), PAI-1 and APA. The presence of Lp(a) and another metabolic antibody increased the risk of PAD symptoms. There was a correlation between the number of altered thrombophilic factors and Fontaine stage of PAD.	B
Tan et al. 2005 23 patients with CLI 36 with IC 30 controls	Platelets, P-sel, PMP	PMPs were increased relatively to healthy controls in patients with IC and further increased in critical limb ischaemia. Platelets and sP-Sel independently predict PAD severity on multivariate analysis	B
Wildman et al. 2005 4787 men and women	CRP, fibrinogen, leucocyte count	On multivariate analysis there were significantly higher odds of developing PAD in the highest compared to the lowest quartiles of CRP, fibrinogen and leucocyte count levels	B
Urge et al. 2005 37 Diabetic patients with PAD	Hct	Increase in claudication distance correlated with haematocrit decrease in diabetic patients with peripheral vascular disease.	C
Makin et al. 2004 20 patients in each group of ischaemic rest pain, acute MI, stable IC, healthy controls	CEC, TF, VWF	Increased number of CECs and elevated TF and VWF in patients with ischaemic rest pain compared to those with IC and healthy controls.	A
Lee et al. 2004 33 PAD patients admitted for	VCAM-1, ICAM-1, P-sel, CRP	VCAM-1, ICAM-1 and CRP were all higher and P-sel significantly lower in patients with systemic arterial disease. P-Sel, VCAM-1,	A, B

elective PTA		hsCRP were elevated significantly for at least 2 weeks post PTA/stenting	
Tseng et al. 2004 557 patients with type II diabetes	Lp(a)	ABPI was associated with log Lp(a) especially in men or in patients with PAD. Lp(a) levels increased from no PAD to mild and severe PAD.	B
Parsson et al. 2004 40 patients with CLI undergoing either femoro-popliteal or femoro-distal reconstruction	tPA, PAI-1, D-dimer, IL-6, IL-2 rec, MCP-1, IL-10, TAT	Patients with CLI had increased IL-6, IL-2 receptor, TAT, tPA, D-dimer and fibrinogen prior to revascularisation. Elevated tPA and D-dimer were found after 30 days posoperatively. Increased IL-6, IL-10, MCP-1 was observed after reperfusion but normalised after 30 days.	B
Riba et al. 2004 39 patients with IC 14 controls	Hcy	63% of patients with PAD were found to be mildly hyperhomocysteinaemic. Claudicants with elevated homocysteine had altered platelet function compared to controls	B
Koscielney et al. 2004 2821 men and women	Hct, plasma viscosity, fibrinogen, erythrocyte aggregation	In males with PAD, Hct, plasma viscosity, fibrinogen and erythrocyte aggregation were significantly higher than controls.	B, C

1.3.4 Circulating markers of endothelial damage/ dysfunction associated with PAD

1.3.4.1 Von Willebrand Factor (VWF)

Von Willebrand Factor is produced in the Wiebel-Palade bodies of endothelial cells, α-granules of platelets and in sub-endothelial connective tissue. VWF is considered as a well-validated plasma marker for the measurement of endothelial damage [Lip et al. 1997] in atherosclerosis and its levels may be regulated by several factors, including blood flow, platelet number, thrombin, angiogenic markers and pro-inflammatory cytokines [Zanetta et al. 2000]. VWF is also associated with the major established cardiovascular risk factors, and modification of these risk factors has been shown to reduce its levels [Blann et al. 1993a; Blann et al. 1993b; Spencer et al. 2002]. VWF is also a carrier for the coagulation factor VIII and VWF promotes thrombus formation by mediating the adhesion of platelets to the sub-endothelium and to each other during haemostasis [Ruggeri 1997].

In the context of PAD, VWF appears to be related to the degree of endothelial damage in diseased lower extremity arteries [Blann et al. 2000]. In PAD, VWF concentration was correlated with transcutaneous oxygen pressure (a marker of severity of limb ischaemia [Seigneur et al. 1995]. VWF has been associated with both progression and severity of disease [Seigneur et al. 1995; Lee et al 1995; Cassar et al. 2005; Cassar et al. 2003] and its elevated levels in patients with critical limb ischaemia have been reported to partially resolve

with resolution of ischaemia [Woodburn et al. 1995], implying some improvement in endothelial function in these patients.

1.3.4.2 Soluble Thrombomodulin (sTM)

Thrombomodulin (TM) is an anticoagulant protein that is specifically expressed on the surface of endothelial cells [Peterson et al. 1999] and is released by these cells in a soluble form (sTM) following endothelial injury. sTM has been shown to be a specific marker of endothelial cell damage [Ishii et al. 1991; Boffa et al. 1991]. Bound TM normally enhances the thrombin-dependent activation of anticoagulant protein C as well as inhibits the pro-coagulant effects of thrombin [Esmon 1995]. The loss of this anticoagulant from the endothelial surface results in a change towards a pro-coagulant state [Blann et al. 1997].

Correlations between TM and Fontaine stage of PAD have been previously reported [Seigneur et al. 1995; Blann et al. 1997] illustrating the progressive release of TM following increasing endothelial injury that occurs as PAD progresses. sTM also rises after exercise in PAD patients and correlates with transcutaneous oxygen pressure [Constans et al. 2000]. The prognostic value of sTM was initially suggested by sTM being the only endothelial marker associated with a significant relative risk for re-stenosis after percutaneous transluminal angioplasty (PTA) in symptomatic PAD patients [Tsakiris et al. 1999]. However, sTM has not been conclusively associated with PAD in all studies. The most notable is the Atherosclerosis Risk in Communities (ARIC) study, which found no association between sTM and PAD in asymptomatic patients [Salomaa et al. 2001]. These conclusions are also supported by other studies [Blann et al. 2000a; Sernau et al. 1995; Peter et al. 1997].

1.3.4.3 Circulating endothelial cells (CECs)

Circulating endothelial cells in the blood are thought to be the product of a disease process irreversibly damaging the endothelium causing endothelial cells to slough off [Blann et al.2005]. This may leave a denuded sub-endothelium exposed to pro-coagulant factors in the blood and thus, may potentiate thrombosis [Lee et al. 2006]. In healthy volunteers CEC numbers have been correlated with thrombomodulin levels [Strijbos et al. 2008]. In disease states, CECs have been correlated with VWF levels and inversely correlated with flow mediated vasodilatation (FMD) suggesting they reflect endothelial cell dysfunction and damage [Rajagopalan et al. 2004]. CECs have also been correlated with tissue factor in pathological disease states linking endothelial damage to on-going thrombogenesis [Rajagopalan et al. 2004]. Elevated CECs may also result in reduced NO release, further amplification of platelet thrombosis and potentiation of atherogenesis [Loscalzo 2001; Freedman et al. 1998].

CEC levels have been reported to be higher in PAD patients with ischaemic rest pain compared to patients with stable claudication, reflecting the irreversible endothelial damage in the more severe manifestations of PAD [Makin et al. 2004]. The prognostic implications of CECs in PAD have yet to be established.

1.3.4.4 Tissue Factor (TF)

Tissue factor (TF) is a cell-surface receptor for coagulation factor VIIa which is expressed on sub-endothelial tissue, platelets and leucocytes, and is a major initiator of thrombogenesis as part of the extrinsic pathway. TF expression is regulated by various cytokines, including tumour necrosis factor (TNF)-α and interleukin (IL)-6. Exposure of the sub-endothelium, either by endothelial injury or by rupture of an atherosclerotic plaque, allows TF to complex with circulating factor VIIa, which via activation of factor X leads to the formation of thrombin and ultimately, formation of a fibrin clot. TF is elevated in prevalent PAD [Makin et al. 2003; Blann et al. 2000b] and associated with disease severity [Makin et al.2004]. TF is yet to be established conclusively as a marker of endothelial damage/ dysfunction and further research is required into its prognostic value in patients with PAD.

1.3.4.5 Cellular adhesion molecules (CAM)

Cellular adhesion molecules (Intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1)) are endothelial ligands for integrins expressed on leucocytes and platelets [Constans et al. 2006]. These molecules facilitate platelet and leucocyte adhesion to the endothelium. Their over-expression causes increased endothelial adhesion of leucocytes and their accumulation in sub-endothelial regions of atheroma [Davies et al. 1993]. Although considered a measurement of endothelial damage/dysfunction, CAMs are not specific to the endothelium and are expressed on a variety of other cell types. CAM expression is up-regulated by atherogenic stimuli and their soluble levels increase in response to inflammatory cytokines and oxidised LDL-C [Constans et al. 2006].

The prognostic value of sICAM-1 and sVCAM-1 in PAD has not been established and there is a suggestion that their association with PAD may be race related [Khalegi et al. 2008]. Nonetheless, elevated sVCAM-1 and sICAM-1 have both been correlated with ankle brachial pressure index, an index of PAD severity [Iwashima et al 2006]. In the Edinburgh Artery Study, sICAM-1 (but not sVCAM-1) was an independent predictor for the development of PAD [Pradhan et al. 2002; Tzoulaki et al. 2007] and symptomatic PAD [Tzoulaki et al. 2007]. There was also a significant trend between higher sICAM-1 levels and the progression of PAD over 17years, from no disease at baseline, to moderate severity (intermittent claudication) and severe disease (i.e. critical limb ischaemia (CLI) or surgical intervention) [Tzoulaki et al. 2007]. Other studies have reported raised sVCAM-1 but not

sICAM-1 that correlated with the extent of peripheral atherosclerosis [Peter et al. 1997] and a higher cardiovascular event rate in symptomatic PAD subjects [Silvestro et al. 2005]. In one study on Fontaine II-IV PAD patients, higher sVCAM-1 but not sICAM-1 levels were also seen at two weeks post percutaneous transluminal angioplasty (PTA) [Lee et al. 2004] and correlated with re-stenosis following PTA [Taskiris et al. 1999].

1.3.4.6 Abnormal structural indices

Various structural indices can be easily quantified in individuals using ultrasound imaging techniques, whereby abnormal measures such as carotid intima medial thickness (IMT and arterial stiffness reflect blood vessel abnormalities.

1.3.4.6.1 Carotid artery intima media thickness

Intima-media thickness (IMT) measurement by ultrasonic evaluation is a well-recognised index of pre-clinical atherosclerosis [Allan et al. 1997] and a predictor of future cardiovascular events [O'Leary et al. 1999]. IMT can be measured at a single site or several sites in the common carotid, carotid bifurcation and internal carotid arteries, but it is unclear whether generalised IMT or focal plaque formation is of more importance in determining cardiovascular risk.

Common carotid IMT has been significantly correlated with the conventional cardiovascular risk factors, such as smoking, diabetes mellitus and LDL-C [Markus et al. 2001; Mohan et al. 2000]. Only C-reactive protein (CRP) and fibrinogen seem to be unequivocally related to IMT [Baldassarre et al. 2008].

Studies in dyslipidaemic and diabetic patients have reported that a combination of carotid IMT and Framingham Risk Score improved the prediction of subsequent cardiovascular

events, better than using the Framingham Risk Score alone [Baldassarre et al. 2007; Bernard et al. 2005]. CCIMT is thought to be an independent risk factor for cardiovascular events in European high risk individuals over and above Framingham risk factors [Baldessarre et al. 2012]. Baldessarre et al. 2007 reported that elevated maximum CCIMT significantly improved the predictive value of Framingham risk score in subjects having a high Framingham risk score (20-30%) (χ^2 =8.13; p=0.04). Bernard et al. 2005 reported an improvement in risk prediction by addition of CCIMT to Framingham risk score in a Cox model (global χ^2 increased from 14.1 to 18.1, P = 0.035). A recently published multi-centre study on 3703 subjects followed up over a median of 36.2 months investigating whether IMT adds to the predictive accuracy of Framingham risk factors [Baldassarre et al. 2012] has shown that maximum CCIMT measurement has a net reclassification improvement of 11.9% over Framingham risk factors alone ($p<0.01$). When this measurement combined with inter-adventitia common carotid artery diameter had a net reclassification improvement of 19.9% ($p<0.01$). Compared to classification based upon Framingham risk factors alone, a combination of CCIMT (mean and maximum) and inter-adventitia common carotid artery diameter had a net reclassification improvement of 12.1% ($p<0.01$) [Baldassarre et al. 2012]. This remained significant even after adjustment for pharmacological treatment [Baldassarre et al. 2012].

IMT is associated with both asymptomatic and symptomatic PAD [Allan et al 1997; McDermott et al. 2005; Price et al. 2007]. In PAD subjects, high common carotid IMT is significantly associated with a risk of subsequent cardiovascular events, independent of traditional risk factors [Price et al. 2007]. However, it is not evident from the published

literature whether carotid IMT alone contributes to risk prediction above what is provided by neither traditional cardiovascular risk factors nor the effect of routine IMT measurements on patient outcomes.

1.3.4.6.2 Arterial Stiffness

The elastic property of arteries varies along the arterial tree; proximal arteries being more elastic than the stiffer distal arteries. Normally the systolic pressure wave is transmitted forwards along the more elastic proximal arteries, with the stiffer distal vessels causing resistance to flow and wave reflections [Laurent et al. 2006]. Branches and bifurcations along the vessel also contribute to reflected pressure waves. With age, and atherosclerosis-associated accumulation of arterial calcium and elastin, there is decreased elasticity and compliance of proximal arteries [Chobnanian 2007], although arterial stiffening with age does not appear to depend on the presence of atherosclerotic diseases [Avolio et al. 1985]. Arterial stiffness may therefore be considered both an index of endothelial dysfunction and an index of abnormal blood flow.

Indices of arterial stiffness include pulse wave velocity and aortic augmentation index (AIx). Arterial stiffness has been reported to be an independent predictor of cardiovascular mortality [Laurent et al. 2001]. Increased arterial stiffness and/ or pulse wave reflections are both associated with the conventional cardiovascular risk factors such as obesity, smoking, hypertension, hypercholesterolaemia and diabetes mellitus, along with novel risk factors, including hyperhomocysteinaemia and raised CRP [Laurent et al. 2006].

The elastic properties of large and small arteries are reduced in PAD patients compared to controls [Kals et al. 2006; Tai et al. 1999]. The arterial pulse wave form is also altered in

these patients [McVeigh et al. 1997] suggesting altered vascular tone in these patients. Arterial stiffness may be improved by aerobic exercise, possibly due to improved endothelial function or by anti-inflammatory and antithrombotic effects [Seals et al. 2008]. AIx is reported as being independently associated with lower ABPI [Khaleghi et al. 2007] along with a reduced walking distance in subjects with PAD [Brewer et al. 2007]. As with other novel risk factors, arterial stiffness has not yet shown any additional benefit over and above traditional risk factors in determining prognosis in patients with PAD. Arterial stiffness may in fact be a consequence of cardiovascular risk factors, age and arterial disease rather than being an independent risk factor in the development of PAD.

1.3.5 *"Abnormalities of blood constituents"* and Peripheral Arterial Disease

Many blood constituents have been implicated in PAD development, progression and prognosis along with acute thrombotic complications. Examples of studies reporting associations between these factors and PAD are shown in table 1.3.

1.3.5.1 Platelets

Important thrombogenic platelet components include α-granule constituents (VWF, fibrinogen, factor V, thrombospondin, β-thromboglobulin), and an adhesion molecule expressed on α-granule membranes, P-selectin. The latter is transferred to the plasma membrane through membrane fusion after platelet stimulation and activation and hence, has been used as an index of platelet activation [Rajagopalan et al. 2007]. Cardiovascular risk factors, including smoking, dyslipidaemia, hypertension and diabetes mellitus all cause chronic endothelial injury, thus stimulating CAM expression, and resulting in increased platelet adhesion to the endothelium [Figure 1.2]. Acute endothelial injury or rupture of a complicated plaque leads to exposure of the sub-endothelium and binding of platelets via sub-endothelial bound VWF thus potentiating thrombosis. Altered shear stress states also induce platelet activation, aggregation and microparticle formation [Holme et al. 1997; Kroll et al. 1996; Nomura et al. 1997] further potentiating thrombogenesis.

Adverse indicators of platelet function in PAD have been reported, including increased β-thromboglobulin [Cella et al. 1979], increased platelet aggregation [Robless et al. 2003;

Kudoh et al. 2006], increased fibrinogen binding [Cassar et al. 2003], increased platelet P-selectin and sP-sel [Ridker et al. 2001; Koksch et al. 2001], and an increase in platelet microparticles [Tan et al 2005]. Platelet and sP-sel levels also independently predict disease severity in PAD [Tan et al 2005]. In another study, symptomatic subjects undergoing PTA, demonstrated a correlation between sP-sel and re-stenosis at follow up [Taskiris et al. 1999].

1.3.5.2 Fibrinogen

Fibrinogen is an important component of the coagulation system, as a pre-cursor of fibrin, and a major determinant of plasma viscosity. Fibrinogen plays an important role in platelet aggregation and mediates the adhesion of platelets to the endothelium via binding with ICAM-1 on its surface [Kamath et al. 2003]. Fibrinogen also plays a major role in inflammation by facilitating a chemotactic response via increased leucocyte adherence to the endothelium [Kamath et al. 2003]. High fibrinogen levels have been associated with smoking, diabetes, LDL-C and obesity, and levels are inversely correlated with HDL-C, alcohol use and physical activity [Kamath et al. 2003].

Fibrinogen has long been recognised as having prognostic implications for cardiovascular mortality. A meta-analysis reported moderately strong associations between fibrinogen levels and risk of coronary heart disease, stroke and other vascular (and non-vascular) mortality [Fibrinogen Studies Collaboration 2005]. The mechanism(s) for this relationship are unknown but hyperfibrinogenaemia increases platelet aggregation, as well as providing a ready source of fibrin. Increased fibrinogen levels favour a higher rate of fibrin-formation, thus leading to a tighter gel structure that most likely is more thrombogenic than structures formed in lower fibrinogen levels [Blann et al. 2001].

Hyperfibrinogenaemia has been associated with prevalent PAD, disease severity and with increased mortality risk in this disease [Ridker et al.2001; Price et al. 1999; Wattanakit et al.

2005; Lip et al. 2002; Vene et al. 2003]. Raised pre-interventional levels of fibrinogen are related to a greater risk of peripheral re-stenosis following peripheral PTA [Schillinger et al. 2002; Roller et al. 1999], with patency rates being significantly associated with fibrinogen levels, independent of other factors [Laxdal et al. 2006]. Importantly, a report on the 17 year follow up to the Edinburgh Artery Study reported strong associations between fibrinogen and incident PAD over 17 years [Tzoulaki et al. 2007] independent of traditional cardiovascular risk factors and cardiovascular disease (defined by a history of MI, stroke or angina) at baseline. Another recent study reported fibrinogen to be a predictor of all-cause mortality risk in PAD [Bartlett et al. 2009] independent of traditional cardiovascular risk factors. However, fibrinogen may not improve the predictive ability over and above traditional cardiovascular risk factors and ABPI for PAD [Tzoulaki et al 2007; Bartlett et al. 2009]. Tzoulaki et al. 2007 investigated the incremental benefit of considering inflammatory, haemostatic and rheological markers in addition to traditional cardiovascular risk factors and ABPI to discriminate incidental symptomatic PAD cases by calculating the area under the ROC curve. The area under the ROC curve in the core model using traditional risk factors and ABPI was 76.1% (95% CI: 71.1-81.3). When fibrinogen was entered into the model this increased to 77.4% (95% CI: 73.6-81.2) [Tzoulaki et al. 2007]. Despite the correlations between fibrinogen and cardiovascular risk, there is no convincing evidence to show that lowering the fibrinogen levels will result in a significant reduction in risk.

1.3.5.3 Fibrin D-dimer

Fibrin D-dimer is a measurable breakdown product of cross-linked fibrin, and marks ongoing intravascular thrombogenesis and abnormal fibrin turnover. It is a specific marker of fibrinolysis but also reflects the severity of a hypercoagulable state [Matsuo et al. 2000]. D-dimer has been reported to induce the synthesis and release of inflammatory cytokines [McDermott et al. Circulation 2003] and has a significant association with cardiovascular diseases and risk factors [Danesh et al. 2001; Lassila et al. 1993]. With reference to PAD, high D-dimer levels have been associated with disease severity [Lee et al. 1995; Tzoulaki et al 2007] and functional impairment [McDermott et al.2003] but was not significantly associated with the development of [Tzoulaki et al. 2007] or progression of disease [Musicant et al. 2006; Mota et al. 2008]. In claudicants, however, raised D-dimer levels do predict fatal and non-fatal coronary events [Ridker et al. 1994]. Further research is required to determine whether lowering D-dimer levels has any effect on cardiovascular outcomes.

1.3.5.4 Fibrinolysis

The fibrinolytic system consists of the circulating pro-enzyme plasminogen, which on activation to produce plasmin by tissue plasminogen activator (tPA) and urokinase, promotes fibrinolysis [Reiner et al. 2001]. tPA a serine protease, normally found on the endothelial cell surface, is secreted following vascular injury. Fibrinolysis is inhibited by the pro-coagulant factor plasminogen activator inhibitor (PAI)-1, a serine protease inhibitor [Philipp et al. 1997] and hence is a marker of impaired fibrinolysis and atherothrombosis. PAI-1 is synthesised by a number of different cells, but it is endothelial derived PAI-1 that is primarily responsible for its levels measured in the plasma [Sobel 1999; Loskutoff et al. 1993].

Endothelial dysfunction results in activation of endothelial cells, generating an imbalance between tPA and PAI-1, which creates a pro-coagulant surface. Although these factors are found at the endothelial surface, they are usually measured as markers of fibrinolysis and not as indices of endothelial dysfunction per se [Felmelden et al. 2005].

Elevated plasma PAI-1 decreases fibrinolysis and enhances thrombosis, and antibodies directed against PAI-1 prevent the progression of thrombosis [Philipp et al. 1997]. PAI-1 has also been detected in the intima of atheroma, thus supporting its role in the pathogenesis of this condition [Philipp et al. 1997]. Plasma PAI-1 is influenced by a number of hormones and cytokines [Cessari et al 1999] and has been associated with hyperglycaemia,

hypertriglyceridaemia and insulin resistance [Reiner et al. 2001; Devaraj et al. 2003], which are all components of the metabolic syndrome. Both tPA and PAI-1 have previously been associated with prevalent PAD and severity of disease and PAI-1 activity has been correlated with re-stenosis after PTA [Roller et al. 1999; Sawa et al. 1994]. Although tPA was not associated with incident PAD in the Edinburgh Artery Study, elevated tPA levels have been associated with the presence of and increasing severity of PAD [Tzoulaki et al. 2007].

1.3.5.5 Lipoprotein (a)

Lipoprotein (a) (Lp(a)) is a large protein molecule that consists of two components: an LDL-like particle and an attached apolipoprotein(a) (apo(a)) [Kronenberg et al. 1999]. Due to its structural similarity to plasminogen, Lp(a) inhibits plasminogen binding to fibrin and endothelial cells by inhibiting tPA [Loscalzo et al. 1990], and therefore fibrinolysis (and promoting thrombosis) [Paraskevas et al. 2008; Palabrica et al. 1995]. Lp(a) accumulates in atheroma and may impair endothelial function and induce smooth muscle proliferation [Reiner et al. 2001]. Little research into the prognostic benefit of Lp(a) in PAD exists. Lp(a) has been reported to be an independent risk factor for PAD [Cheng et al. 1997] as well as severity of disease [Cheng et al. 1997; Tseng et al. 2004].

1.3.5.6 Inflammatory factors

Inflammation plays a major role in all stages of atherogenesis [Figure 1.2] [Ross 1999; Stary et al. 1995]. Inflammation occurs in response to a variety of stimuli and is also associated with many traditional cardiovascular risk factors, including dyslipidaemia, hypertension, diabetes mellitus, obesity and infection [Ross 1999; Libby et al. 2002] [Figure 1.2].

Of the wide range of inflammatory indices, IL-6 and CRP have probably been most investigated. IL-6 is a pro-inflammatory cytokine that induces a prothrombotic state by increasing expression of fibrinogen, TF, Factor VIII and VWF [Tzoulaki et al. 2007]. IL-6 also activates endothelial cells and their adhesiveness by up-regulating E-selectin, ICAM-1 and VCAM-1, thus leading to increased leucocyte-endothelial binding and increasing platelet production [Tzoulaki et al. 2007]. CRP is a circulating acute phase protein synthesised by the liver, and its release is stimulated by IL-6 and other pro-inflammatory cytokines along with promoting monocyte chemotaxis and TF expression [Devaraj et al. 2003].

CRP has pro-atherogenic effects on all cellular components of the endothelium. It inhibits endothelial cell NO synthase resulting in reduced bioavailability of NO and decreased endothelial dependent vasodilatation. CRP increases expression of ICAM-1 and VCAM-1, whose effects are discussed above, and increases production of PAI-1, which inhibits fibrinolysis [American Diabetes Association 2003; Vinik et al. 2001].

IL-6 and CRP are associated with PAD development, progression and severity of disease [Ridker et al. 2001; Devaraj et al. 2003; Tzoulaki et al. 2005; Allison et al. 2006, Armitage et al. 2006; Riba et al. 2004]. Elevated CRP levels are also associated with functional impairment [McDermott et al.2003] and increased thrombotic complications in symptomatic PAD [Hogj et al. 2008]. In the Edinburgh Artery Study, CRP was one of the few markers significantly associated with PAD after 17 years follow-up, even after adjusting for cardiovascular risk factors [Tzoulaki et al. 2007]. As with fibrinogen, Lp(a) and haematocrit, CRP provided very little prognostic information for incident PAD to that obtained by cardiovascular risk factors and ABPI [Tzoulaki et al. 2007]. IL-6 only showed weak associations and were attenuated when these risk factors were accounted for [Tzoulaki et al. 2007].

1.3.5.7 Homocysteine

Homocysteine is a highly reactive, sulphur containing amino acid formed as a by-product of methionine metabolism. Adverse effects of homocysteine include vascular endothelial injury [Wall et al. 1980], increased adhesion molecule expression [Silverman et al. 2002] smooth muscle proliferation, and oxidation of LDL-C, which contributes to a prothrombotic vascular endothelial microenvironment [Moghadasian et al. 1997; Welch et al. 1998].

Homocysteine has been associated with an increased risk of PAD and lower ABPI measurements in a previous meta-analysis [Boushey et al. 1995] but this finding has been disputed [Taylor et al. 2003]. In the Multi-Ethnic Study of Atherosclerosis, homocysteine was significantly associated with PAD, even after adjustment for traditional risk factors [Allison et al. 2006]. Elevated homocysteine levels have also been associated with lower patency rates following revascularisation procedures and lower mean amputation free survival [Heneghan et al. 2008]. As homocysteine levels increase following an acute thrombotic event, it is difficult to know whether increased thrombosis is due to elevated homocysteine or vice versa. This may be the reason why a link between elevated homocysteine and PAD has not been conclusively confirmed.

1.3.6 *"Abnormal Blood Flow"* and Peripheral Arterial Disease

Quantification of the flow properties of blood can be made by measuring haemorrheological indices, and by measuring wall shear stress. Examples of studies reporting positive associations with PAD can be found in table 1.3.

1.3.6.1 Haemorrheological indices

Haemorrheological indices that have been investigated previously in PAD include blood viscosity (influenced by erythrocytes, leucocytes and platelets) and plasma viscosity and its determinants, including fibrinogen, VWF and lipoproteins [Tzoulaki et al. 2007; Fibrinogen Studies Collaboration 2005; Lowe et al. 1993]. Haematocrit, blood viscosity, plasma viscosity and fibrinogen have each been reported to be significantly related to the severity of PAD; for example, blood viscosity and fibrinogen remained significantly associated with ABPI on multiple regression analysis [Lowe et al.1993]. Blood viscosity and its determinants are also correlated with CCIMT, in a linear fashion [Lee et al.1998]. In the Edinburgh Artery Study, all rheological markers were significantly increased at baseline in all subjects who developed PAD over 17 years follow up [Tzoulaki et al. 2007]. Plasma viscosity is an independent risk factor for progression of atherosclerosis in claudicants [Smith et al. 1998]. The effect of haemorrheological indices on PAD in the Edinburgh Artery Study was modest and was considerably reduced after adjusting for traditional risk factors [Tzoulaki et al. 2007]. These indices alone are therefore unlikely to offer additional clinical value in PAD risk prediction.

1.3.6.2 Wall Shear stress (WSS)

WSS is the force that contrasts friction applied to the blood by the vascular wall [Van Der Loo et al. 2005]. Its two components are shear rate (the rate at which adjacent layers of fluid move with respect to each other) and blood viscosity (the capacity of the blood to offer resistance to flow) [Gori et al. 2007]. Important determinants of WSS are geometric factors, such as bifurcations, tortuosity and aneurysms, as well as various biological and systemic factors, such as systolic blood pressure and NO [Gori et al. 2007; Shaaban et al. 2000].

Both the synthesis and release of various prothrombotic and pro-inflammatory mediators and the secretion and release of endothelial defences (e.g. NO and prostacyclin) are shear dependent [Lowe 2003/2004]. Specific arterial sites, such as branches, bifurcations and curvatures cause specific alterations in the flow of blood, resulting in decreased WSS and increased turbulence [Ross 1999]. Decreased WSS is associated with decreased NO synthase production, reduced endothelial cell repair, increased reactive oxygen species (ROS), increased endothelial cell permeability to LDL-C, increased leucocyte adhesion (via ICAM-1 and VCAM-1), and an increase in apoptosis and smooth muscle proliferation [Cunningham et al. 2005; Niwa et al. 2004; Chiu et al. 2004; Walpola et al. 1995]. The impaired normal laminar shear stress that occurs at bifurcations and branches may reduce local production of endothelial-derived NO leading to less endogenous atheroprotective mechanisms at these sites [Libby et al. 2002]. These sites are therefore more susceptible to atherosclerosis.

WSS is associated with traditional risk factors, but only smoking, age and triglycerides remained significantly associated with this on multivariate analysis [Spring et al. 2006]. An

inverse relationship that exists between WSS and CCIMT has been reported [Irace et al. 2004]. The IMT in normal abdominal aorta necropsy specimens from young adults correlated significantly with mean, minimum and oscillatory WSS [Asakura et al. 1990; Gnasso et al. 1997; Zarins et al. 1993], suggesting a role of this marker even in the early stages of atherogenesis. Common carotid artery WSS is a local risk factor for PAD and is reduced in patients with symptomatic disease [Van Der Loo et al. 2005, Spring et al. 2006] and aneurysms [Spring et al. 2006].

1.3.7 Summary of Virchow's Triad and Peripheral Arterial Disease

The pathophysiological processes involved in PAD and its symptomatic manifestations are atherogenesis and thrombogenesis. There is evidence that abnormalities in the three components of Virchow's Triad are related to the severity and prognosis of PAD. Subjects afflicted with this common disease have a high morbidity and mortality from atherothrombotic events. This may partly be explained by the on-going endothelial and enhanced coagulation activation that occurs in these subjects causing a hypercoagulable or pro-thrombotic state. Attention to this state may possibly provide answers to the future management of this condition.

1.4 Angiogenesis and Peripheral Arterial Disease

1.4.1 Introduction

Angiogenesis is the formation of new blood vessels that results from stimulation of endothelial cells by vascular growth factors. Pathological disruption of this process is a hallmark of vascular overgrowth or vascular insufficiency. Angiogenesis is initiated by a number of different stimuli, including hypoxia, inflammation, mechanical factors such as wall shear stress and stretch. These stimuli, either directly or indirectly, activate endothelial cells by initiating autocrine or paracrine production and release of growth factors and cytokines. The angiogenic process is complex and not completely understood, but a number of growth promoting factors have been found to regulate the induction of angiogenesis. Of the numerous angiogenic markers identified, several have been associated with PAD, some also being investigated with regards to their therapeutic application.

Angiogenesis is frequently observed in atherosclerotic lesions [Post 2008] and is a feature of development and progression of disease [Jeziorska 1999; Moreno 2004]. This process is driven by mediators, including vascular endothelial growth factor (VEGF), angiogenin and angiopoietins, produced by a number of different cells in a variety of conditions [Caine et al. 2006]. Angiogenesis is especially frequent in advanced disease activity and may thus characterise atherosclerotic lesions at high risk of haemorrhage or rupture [Kolodgie 2003; Moreno 2004, McCarthy 1999; Mofidi 2001].

1.4.2 Vascular Endothelial Growth Factor (VEGF)

VEGF is a mitogen and is perhaps the most important growth factor promoting endothelial cell proliferation and angiogenesis. VEGF is expressed in virtually all vascularised tissues and is secreted in the vascular wall by endothelial and smooth muscle cells. It has been suggested that low physiological VEGF levels are needed for the maintenance of vascular homeostasis [Maharaj et al. 2006]. Pathological states, such as hypertension, hypercholesterolaemia and atherosclerosis also up-regulate VEGF expression [Makin et al. 2003], therefore its levels are increased in these conditions.

Hypoxia is known to be one of the stronger inducers of angiogenesis, and is perhaps the strongest influence of VEGF and expression of its receptors (Flt-1 ((FMS-like tyrosine kinase-1 or VEGF-receptor-1)) and Flk-1 (Fetal liver kinase-1 or VEGF-receptor-2))) and other growth factors in PAD, including fibroblastic growth factors (FGF) 1 and 2 and platelet derived growth factor (PGDF). VEGF transcription is also influenced by hypoglycaemia and acidosis and is further stimulated by other growth factors, including FGF-2, PDGF-2, transforming growth factor- beta (TGF-β) and Placental growth factor (PlGF). The ischaemic environment which exists in PAD causes binding of hypoxia inducible factor 1-α to the hypoxia response area in the VEGF gene promoter region. The increased VEGF which ensues, after binding to Flk-1, causes a tyrosine kinase signalling cascade in endothelial cells that stimulates production of other growth factors that variously stimulate vessel permeability, proliferation and survival, migration and finally differentiation into mature blood vessels.

VEGF has been demonstrated to be increased in patients with PAD [Makin et al. 2003; Roller et al. 2001; Belgore et al. 2001] and has been implicated in the progression of atherosclerosis [Khuraana et al. 2005; Cucina et al. 2003; Inoue et al. 1998]. VEGF was found to have the greatest expression in more advanced atherosclerotic arteries compared to early lesions and normal controls, representing a more hypoxic environment which exists in these subjects.

1.4.3 Angiopoietins

The angiopoietins (Angiopoietin (Ang)-1 and Ang-2) are angiogenic growth factors specific for endothelium. Ang-1 and Ang-2 are ligands for the Tie-2 receptor and contribute to the regulation of vascular permeability, inflammation and angiogenesis [Chon et al. 2004, Van der Heijen et al. 2010, Jones et al. 2001]. Cleavage of the extra-cellular domain of Tie-2 by endothelial cells occurs in response to inflammation and results in circulating sTie2, a process which is reduced by hypoxia [Van der Heijen et al. 2010]. In acute inflammatory states, sTie2 in the plasma is bound by Ang-1 preferentially over Ang-2 due to its higher affinity for the receptor, leading to greater binding of Ang-2 to endothelial Tie-2 and increasing endothelial permeability [Ven der Heijen et al. 2010]. In the presence of hypoxia there is less cleavage of Tie-2 from the endothelial surface, less plasma binding of Ang-1/sTie-2, leading to preferential Ang-1/Tie-2 endothelial binding to Ang-2, which accelerates vessel maturation [Post et al. 2008].

Ang-1 acts in conjunction with and potentiates the effects of VEGF in the initiation and acceleration of the process of angiogenesis, being involved in both endothelial cell recruitment and proliferation. Ang-2 is a naturally occurring antagonist for both Ang-1 and the Tie-2 receptor. It is expressed only at sites of vascular remodelling. Ang-2 in the presence of VEGF, promotes a rapid increase in capillary diameter, remodelling the basal lamina and new vessel growth, evidenced by sprouting of existing blood vessels. In contrast, if VEGF is inhibited, Ang-2 leads to endothelial cell death and vessel degradation.

Levels of circulating Ang-1 and Ang-2 have been shown to be raised and associated with cardiovascular risk in patients with PAD [Findley 2008] and other manifestations of atherosclerosis, including heart failure, acute coronary syndromes and hypertension [Felmelden et al. 2003a; Felmelden et al. 2003b; Nadar et al. 2005; Lim et al. 2004; Chong et al. 2004; Lee et al. 2004].

1.4.4 Angiogenin

Angiogenin is a small polypeptide implicated in angiogenesis. Angiogenin may function as a tRNA-specific ribonuclease and has been thought to be an indicator of endothelial damage related to progression of vascular disease. Angiogenin binds actin on the surface of endothelial cells and its plasma levels are dependent on the presence of an angiogenic stimulus [Tello-Montoliu et al. 2006]. Its levels would therefore be expected to be associated with sTie2 and angiopoietins. Once bound angiogenin is endocytosed and translocated to the nucleus thereby promoting the endothelial invasiveness necessary for angiogenesis. Angiogenin is normally found in the vasculature but in some physiological and pathological conditions, including PAD [Burgmann et al. 1996], its levels increase in blood, stimulating endothelial cells to produce new vessels [Bond et al. 1990]. Several studies have suggested that angiogenin and other angiogenic factors could promote atherosclerosis and potentially de-stabilise plaques by promoting intra-lesional angiogenesis [Moreno et al. 2004, Khurana et al. 2005].

Elevated angiogenin levels have been found to be higher in those with severe PAD, compared to mild and moderate disease [Burgmann et al. 1996]. Therefore angiogenin could potentially be an indicator of endothelial damage related to progression of vascular disease.

1.4.5 Angiogenesis and Thrombogenesis

Over the past decade there has been increasing investigation into the association between angiogenesis and thrombogenesis. The importance of TF in thrombogenesis has been described above. Most of this research has been in the field of tumour growth and metastasis. For example, increases in TF have been found to be an indicator of both hepatic metastasis and prognosis in colorectal carcinoma and the expression of TF in sarcoma cells, up-regulates the VEGF gene [Abe et al. 1999] and treatment of endothelial cells with VEGF leads to the up-regulation of tissue factor mRNA and its subsequent expression at the cell membrane [Camera et al. 1999]. The regulation of the TF gene is controlled by several transcription factors activated by inflammatory cytokines (IL-1β and TNF-α), oxidised LDL-C and endotoxin. In vitro, angiogenic growth factors, e.g. PDGF, FGF and transforming growth factor beta or epidermal growth factor can induce TF expression in fibroblasts and smooth muscle cells. This further supports an association between thrombogenesis and angiogenesis.

1.4.6 Angiogenesis and Atherogenesis

The process of atherogenesis has been described above. The link between angiogenesis and atherogenesis is apparent in that they both involve the endothelium. Whilst VEGF may play a role in vascular disease progression by initiating and propagating angiogenesis, VEGF has also been found to progressively increase in atherogenesis; though this is likely to be secondary to hypoxia and inflammation in growing lesions.

By increasing vascular permeability, VEGF may provide an easier passage for inflammatory cells migrating into the developing atheroma within the intima and media [Dvorak et al. 1995]. Factors promoting inflammation cause chronic endothelial injury impairing normal endothelial function, the first established step in the atherogenic pathway. Inflammation and inflammatory markers have previously been found to stimulate FGF and VEGF expression following myocardial necrosis [Sunderkotter et al. 1991a; Sunderkotter et al.1991b]. VEGF can also stimulate and recruit other macrophages to stimulate the inflammatory response further, which further stimulates angiogenesis.

In vitro research has shown co-localisation of Ang-2 and VWF exclusively in Wiebel-Palade bodies, the primary storage granule of VWF in endothelial cells. VWF and Ang-2 export parallel each other, following the same temporal kinetic and may imply co-regulation making them functionally related [Fiedler et al. 2004; Hannah et al. 2002; van den Eijnden-Schrauwen et al. 1997].

Another example of the link between angiogenesis and atherogenesis is the elevated numbers of vaso-vasorum found in atherosclerotic plaques. Vaso-vasorum derived microvessels do not extend to the intima of normal arteries, penetrating only the adventitia and outer media [Geiringer 1951]. As the vessel wall thickness increases in the setting of vascular disease, proliferation of vaso-vasorum and intimal neo-angiogenesis is observed. In fact, intimal neo-vascularisation has been reported as being an almost ubiquitous feature of atherosclerotic disease, correlating with both histological grade and symptoms [Chen et al. 1999; Fleiner et al. 2004]. It is thought that this process is the consequence of hypoxia within the vessel wall. As vascular disease progresses, it is perhaps the ensuing increase in hypoxia that is responsible for the increase in microvessel density within the atheroma. Indeed plaque angiogenesis is associated with more rapidly progressive and unstable vascular disease [Moulton 2002].

1.4.7 The Endothelium: A link between angiogenesis, atherogenesis and thrombogenesis

The three processes of angiogenesis, atherogenesis and thrombogenesis are intrinsically linked together in that they all affect and take place within the endothelium. Even before vascular disease is apparent, cardiovascular risk factors are at play in these pathophysiological processes. By reducing the bioavailability of NO and through production of ROS increasing oxidative stress and reducing flow mediated vasodilatation and increasing shear stress, various cardiovascular risk factors potentiate a thrombogenic and atherogenic microenvironment. This is exemplified by elevations in TF, platelet activation and aggregation, hyperfibrinogenaemia and disordered fibrinolysis. The pro-atherogenic effects of cardiovascular risk factors include the promotion of inflammation causing chronic endothelial activation and injury, resulting in dysfunction and damage. This is illustrated by elevations in VWF, TM and cellular adhesion molecules. As mentioned above, increases in oxidative stress resulting from hypoxia, and continued inflammation and endothelial activation, stimulates angiogenesis.

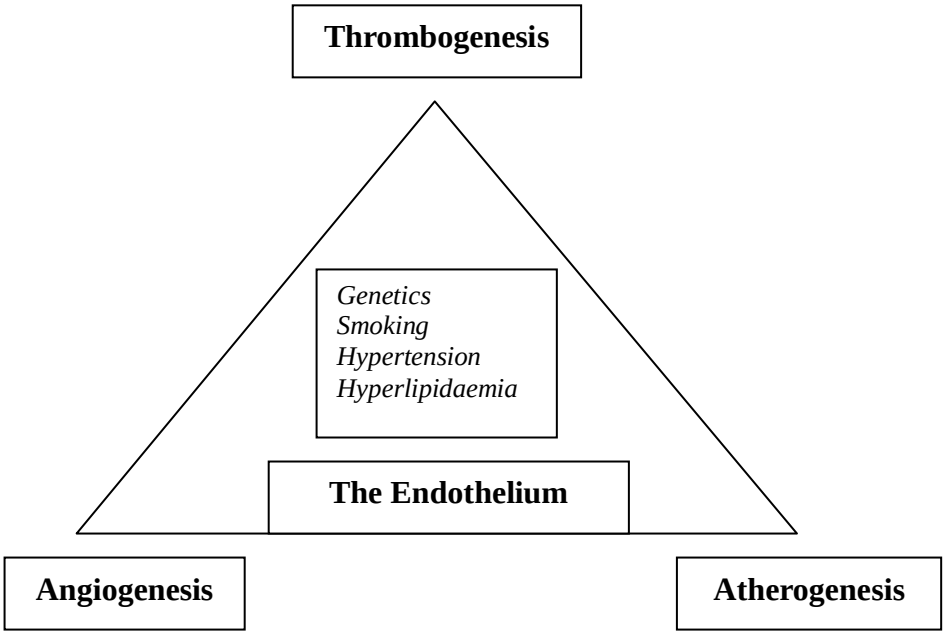
As atheromatous changes progress, along with further increases in VWF and reduction in FMD, subjects develop a coagulopathy with hyperfibrinogenaemia and further platelet irregularities. As patients develop symptomatic disease there is further expression of CAM at the endothelial cell surface and further elevations in VWF (which may reflect normal replacement of dead endothelial cells or release from platelets as they form a thrombus) [Lip et al. 2004].

All of the above processes result in flow irregularities, stenoses and occlusions. Elevated VEGF may reflect the development of collaterals to assist blood flow in these circumstances. It may also reflect the increased numbers of vaso-vasorum in the arterial media or increased vascularity within the atheroma itself. Indeed plaque neo-angiogenesis may be a crucial factor for plaque development and rupture [Celleti et al. 2001], illustrated by the fact than an increase in plaque microvessels has been correlated with more severe disease and plaques with the greatest risk or rupture or haemorrhage.

1.4.8 Summary of Angiogenesis in Peripheral Arterial Disease

A greater understanding of the molecular mechanisms involved in the development and progression of PAD and improvements in biochemical techniques has shown that the processes of angiogenesis, atherogenesis and thrombogenesis are seemingly related. A new vascular triad (the Birmingham 'Vascular Triad' [Figure 1.3] [Lip et al. 2004]), with the endothelium integral, has been proposed.

Figure 1.3: Thrombogenesis, atherogenesis and angiogenesis in vascular disease: the Birmingham 'Vascular Triad' [Lip et al. 2004]



CHAPTER 2

THE PROJECT RATIONALE AND HYPOTHESIS

2.1 Project rationale

Within the UK, the epidemiology of PAD is well documented amongst the white population (e.g. Edinburgh Artery Study [Fowkes et al. 1991]) but there is paucity of literature on other ethnic groups. This represents a major, clinically important gap in knowledge because there is good reason to believe that ethnicity will have an important effect on the prevalence, distribution and severity of PAD.

Ethnic minority groups make up 7.9% of the general population of the UK [Gill et al. 2007] with Asian/Asian British (3.97%) and Black/Black British (1.96%) being the largest of these groups [National Statistics Online]. Previous population based studies in other countries have shown variations in the prevalence of PAD amongst different ethnic groups [Table 1.1]. Limited data on PAD in Indians suggest its prevalence is lower in this group than in Europeans [Premalatha et al. 2000; UKPDS 1994; Mohan et al. 1995; Chaturvedi et al. 2007]. Likewise, US studies have found the prevalence of PAD in African Americans to be higher on the whole than in white group [Allison et al. 2006; Criqui et al. 2005].

A greater understanding of the prevalence and severity of PAD amongst Black and minority ethnic groups in the UK is required for the planning and delivery of clinically and cost-effective treatment of this common condition within an ethnically diverse society. In the

longer term, accurate epidemiologic data will also help target prevention strategies on those at highest risk.

2.2 Primary Aim of Research

To improve the understanding of the epidemiology and pathophysiology of peripheral arterial disease in South Asians (i.e. those originating from India, Pakistan and Bangladesh) and Blacks (i.e. those originating from the Caribbean and sub-Saharan Africa) living in Birmingham, United Kingdom.

2.3 Hypotheses

Hypotheses proposed for this thesis are summarised as follows:

1. Translated versions of the Edinburgh Claudication Questionnaire in the following languages: Urdu, Bengali, Hindi, Punjabi and Gujarati will have equivalent sensitivity and specificity to that found in the Edinburgh Artery Study [Chapter3.1]

2. There will be a difference in prevalence of PAD (Defined by Ankle Brachial Index (ABPI) <0.9) between South Asians and Blacks [Chapter 3.2]

3. Ethnic differences will exist in associations between traditional clinical cardiovascular risk factors (Smoking, diabetes mellitus, dyslipidaemia and hypertension) and PAD in South Asians and Blacks [Chapter 3.2]

4. Amongst South Asians and Blacks ethnic differences will exist in common carotid intima-media thickness, PAD and its association with
    a. traditional cardiovascular risk factors [Chapter 3.3]
    b. circulating markers of inflammation, haemostasis and thrombosis (CRP, IL-6, D-dimer, sP-sel, VWF) and CCIMT and PAD [Chapter 3.4]

5. Ethnic differences will exist in plasma expression of angiogenic markers (Ang-1, Ang-2, Angiogenin, sTie2) between South Asians, Blacks and Whites [Chapter 4.1]

6. Ethnic differences will also exist in the association of angiogenic markers with traditional cardiovascular risk factors and cardiovascular disease [Chapter 4.1]

7. Ang-1, Ang-2, sTie2 and angiogenin levels will be higher in subjects with at least one cardiovascular risk factor compared to healthy controls, with a further increase in those with clear cardiovascular disease [Chapter 4.1]

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2.4 Study Designs

2.4.1 Sub-study to Ethnic Echocardiographic Heart of England Screening (E-ECHOES) Study

2.4.1.1 Aim of sub-study to E-ECHOES

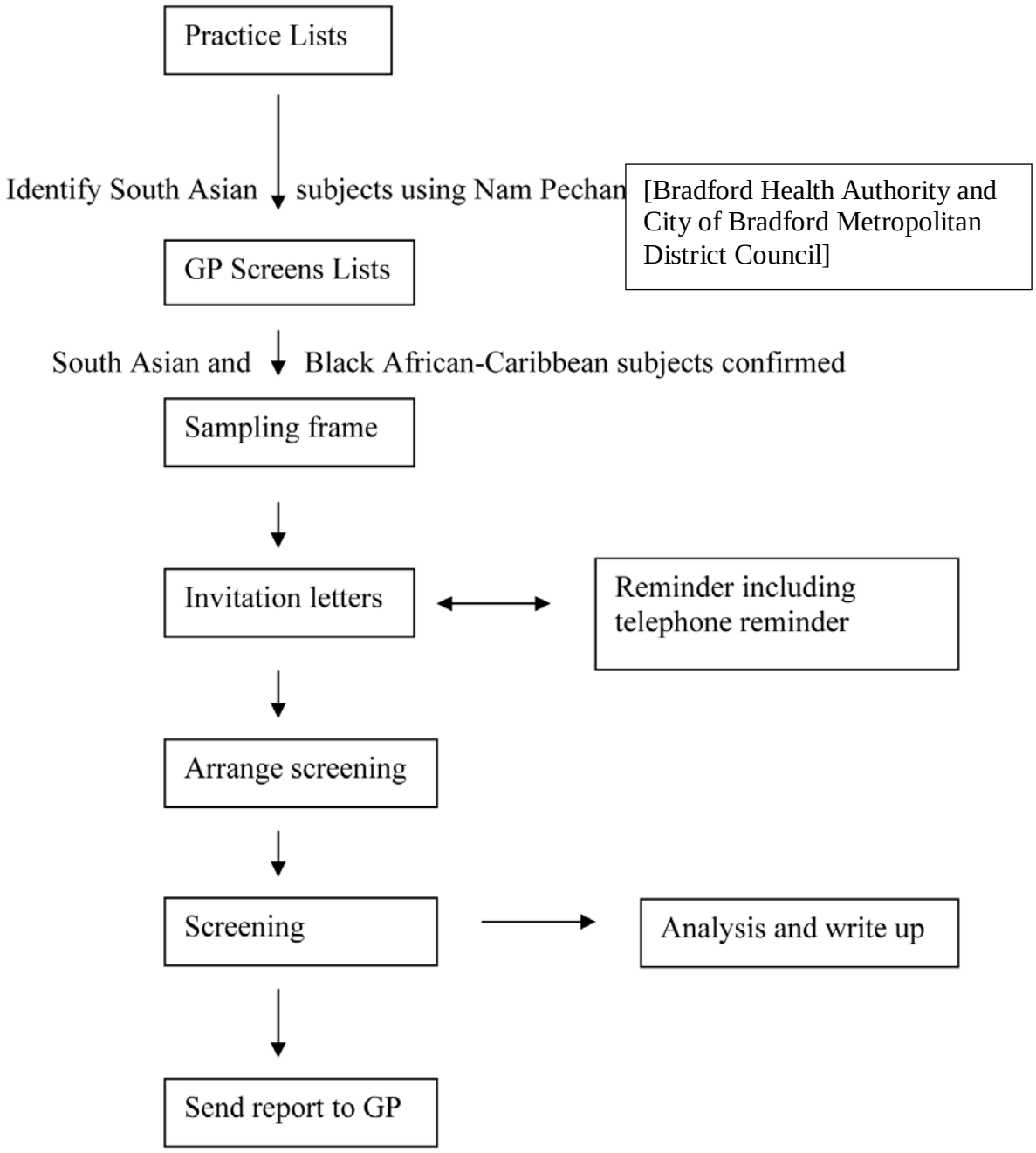
The aim of this study was to establish an estimate of the prevalence of PAD amongst South Asian and Black subjects living in Birmingham, UK and make associations between traditional cardiovascular risk factors, novel risk factors and this disease. Furthermore the aim was to see if there are any ethnic differences in associations between these risk factors and PAD, which might explain any difference in disease prevalence.

2.4.1.2 Participant Recruitment

572 subjects participating in the E-ECHOES Study between March 2008 and February 2009 were recruited into this sub-study. The E-ECHOES [Gill et al. 2009] study is a cross-sectional population survey of a sample of South Asian (i.e. those originating from India, Pakistan or Bangladesh) and Black (i.e. those originating from the Caribbean and sub-Saharan Africa) residents of Birmingham aged 45 years and over. The majority of the South Asian and Black groups in the UK reside in metropolitan areas particularly inner cities such as Birmingham [Gill et al. 2007]. Recruitment was undertaken from September 2006 to August 2009 from 20 primary care centres. This entailed a two-staged process with an initial sample of primary care centres known to have high proportion of these minority ethnic

patients and then a sample using the practice age-sex register. As ethnic group collection is not routinely collected in primary care, multiple methods were used to identify potential subjects. Potential SAs were identified using the Nam Pechan software [Bradford Health Authority and City of Bradford Metropolitan District Council] based upon subject name and visual inspection by Dr Gill [Cummins et al. 1999]; and for Black subjects practice staff were consulted [see Figure 2.1][Gill et al. 2011]. It was important to establish correct ethnic classification of subjects prior to screening as any misclassification may have affected subsequent analyses.

Figure 2.1: Flow of Participants through E-ECHOES study [Gill et al. 2011]



The general practitioner then reviewed the lists to ensure that only South Asian and Black subjects were included and excluded any whom they considered it inappropriate to approach; for example, due to terminal illness or dementia. Potential subjects were mailed an invitation letter, a reminder and telephoned up to 3 times inviting them to participate in the study. All potentially eligible subjects were asked their ethnic group before booking an appointment.

This study complies with the Declaration of Helsinki and the Walsall Local Research Ethics Committee reviewed and approved the protocol (05/Q2708/45). Verbal and written consent was obtained from all participants.

The student was not involved in the initial recruitment process for the E-ECHOES Study. The student was a member of the data collection team for E-ECHOES between March 2008 and February 2009. During this time all patients seen by the student had interviewer-led questionnaire, anthropometric measurements, blood sampling, ABPI and CCIMT measurements performed by the student.

2.4.1.3 Sub-study to E-ECHOES Measurements

To maximise recruitment all eligible subjects were invited to attend for an assessment at their local primary care centre. Data obtained included an interview-administered questionnaire, full physical examination, ECG and echocardiogram. ABPI and CCIMT measurements were undertaken by the student. Interpreters were used as required. Self-reported diagnoses were confirmed with practice medical notes.

The questionnaire included the following data: age; date of birth; address; post code; self-determined ethnicity; religion; place of birth; migration history; languages spoken; level of education; alcohol consumption; cigarette smoking including other tobacco use; exercise assessment; history of illness in self and family; current medication; dyspnoea scoring leading to New York Heart Association functional classification All these measures are based on existing surveys such as the Health Survey for England [Erens et al. 2001] and the Fourth National Survey of Ethnic Minorities in Britain [Modood et al. 1997]. Information on co-morbidity (myocardial infarction, angina, hypertension, heart failure, stroke, diabetes) was obtained.

Subjects were defined as having hypertension if they were previously known to have hypertension from medical records, using anti-hypertensive medication, or whose mean of 3 blood pressure recordings after 5 minutes rest was greater than 140/90. Similarly, subjects were defined as having diabetes mellitus if their medical records stated it or if they were using anti-diabetic medication. Subjects were defined as being illiterate if they never attended school in any country. Former smokers are those who have previously smoked

tobacco but stopped >1 year prior to assessment. Smokers who stopped <1 year prior to assessment were defined as current smokers.

At physical examination, the following measurements were undertaken using standard procedures: Height, weight, body mass index, resting pulse, systolic and diastolic blood pressure using an automated sphygmomanometer (OMRON 715IT), and waist measurement. The height of the JVP was assessed; the heart auscultated for murmurs and added sounds, and the chest examined for signs of congestion and other abnormalities. Hepatomegaly, ascites and peripheral oedema were also examined for. A resting 12 lead ECG (Mortara ELI 150) was recorded.

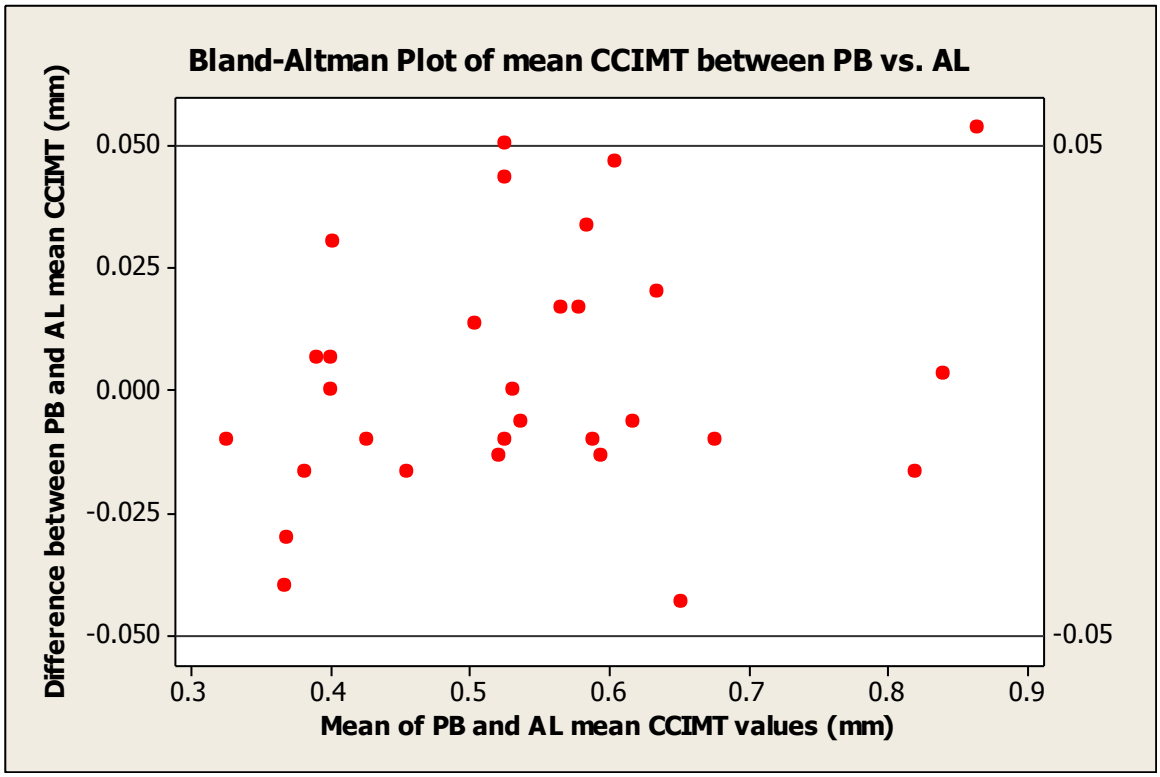
2.4.1.4 Common Carotid Artery Intima Medial Thickness (IMT) Assessment

492 Participants in this sub-study to E-ECHOES had Common Carotid Intima Medial Thickness measured using B-mode ultrasound scanning. All measurements were performed by the student using semi-automated software with the subject supine, using a portable Vivid-i machine (GE Healthcare, Chalfont St Giles, UK). This software has previously been validated in the measurement of IMT [Kanters et al. 1997; Gepner et al. 2000; Stein et al. 2005] and has been shown to be an accurate measure of IMT even with limited training. Each carotid was examined in the transverse and longitudinal scan planes. Measurement of IMT was made from the longitudinal scan plane in a 1cm segment at the point on the far wall of the common carotid artery between 1.5 and 2.5 cm proximal to the bifurcation, with the vessel wall parallel to the transducer face. This showed the intima-medial boundaries most clearly. IMT was measured at this point because the accuracy of visualisation of the intima-medial boundaries is greater and IMT tends to be less variable in the common carotid artery than it is in more distal segments [Folger et al. 1987; Zierler et al. 1987]. A three lead ECG trace was recorded simultaneously with the B-mode images. Three recordings were made in the end-diastolic phase for both the mean and maximum CCIMT on both sides of the neck with a gated ECG. In 7 of the 492 subjects, the student could only measure CCIMT on one side. The mean and maximum CCIMT on the measured side was used in subsequent analyses. In the remaining 485 subjects the side with the largest CCIMT value was used in subsequent analyses.

2.4.1.5 CCIMT reproducibility

Prior to measuring any of the study participants, I underwent training in the measurement of CCIMT using the Vivid-i by a consultant medical physicist and also by attending a Vascular Ultrasound course (Axiom Ultrasound, Imperial College London). Following a period of training, measurements undertaken by me were validated in a study against a consultant medical physicist (AL) familiar with CCIMT measurement, by scanning 30 carotids for mean and maximum CCIMT blinded to the operator's results. Bland-Altman Plots [Bland & Altman 1995] of CCIMT measurements made by me and the consultant medical physicist were analysed. No significant differences were found at a level of 5% between me and the medical physicist's results. The difference between my measurements and the medical physicist's measurements were 1.84% for mean CCIMT and 2.4% for maximum CCIMT, which was considered acceptable. Bland-Altman plots are shown in Figures 2.2 and 2.3.

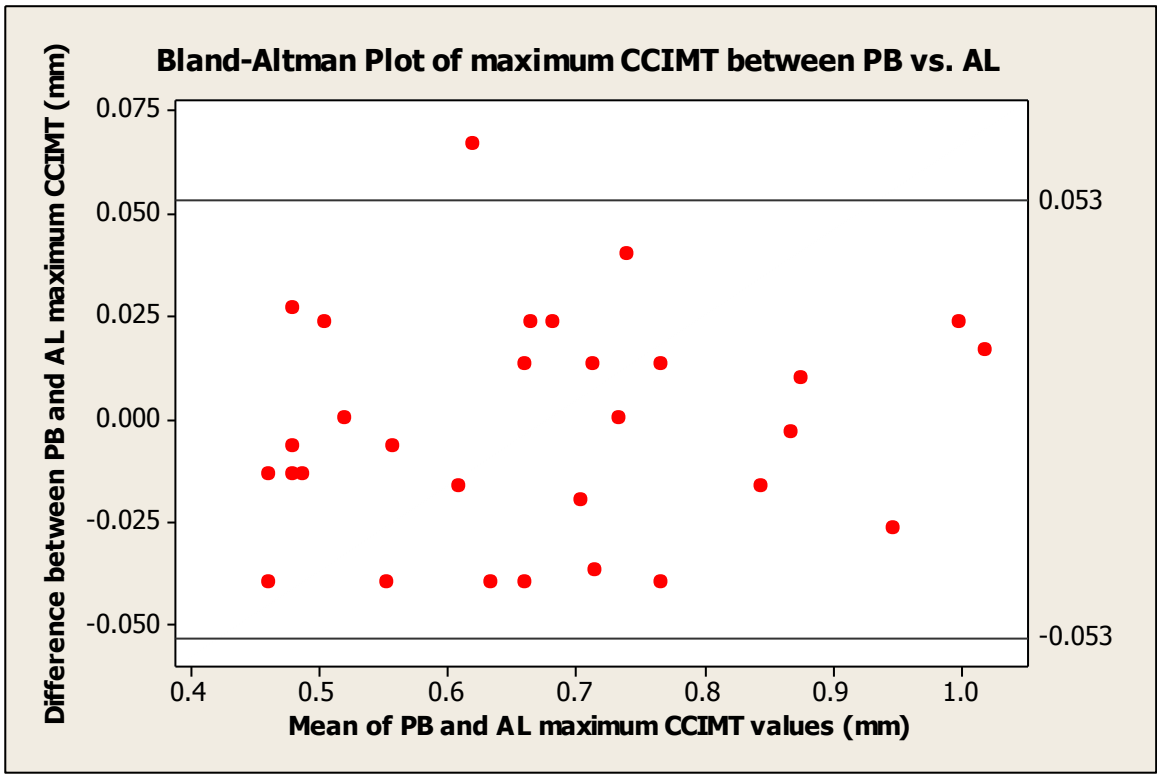
Figure 2.2: Bland-Altman [Bland & Altman 1995] Plot of mean CCIMT between PB and AL



PB: Philip Bennett; AL: Dr Lovick (Consultant medical physicist)
Grid lines showing 95% confidence interval. This value was determined by 1.96 x 0.02529 [Standard deviation] = 0.0496

The difference between my measurements and the medical physicist's measurements were 1.84% for mean CCIMT.

Figure 2.3: Bland-Altman [Bland & Altman 1995] Plot of maximum CCIMT between PB and AL



PB: Philip Bennett; AL: Dr Lovick (Consultant medical physicist)
Grid lines showing 95% confidence interval. This value was determined 1.96 x 0.02709 [Standard deviation] = 0.053

The difference between my measurements and the medical physicist's measurements were 2.4% for maximum CCIMT.

2.4.1.6 PAD Assessment

The presence of PAD was assessed by measurement of Ankle Brachial Pressure Index (ABPI). This was measured after 5 minutes rest in the supine position with a continuous Doppler device (Super Dopplex II, Huntleigh Healthcare), 8 MHz probe and a manual sphygmomanometer. Systolic blood pressure in the brachial artery was measured in both arms using a blood pressure cuff and Doppler detection in the antecubital fossa. Systolic blood pressure was recorded 3 times in each arm. Systolic blood pressure in the left and right dorsalis pedis and posterior tibial arteries was then measured in a blood pressure cuff applied just proximal to the malleoli. For each pressure measurement, the pulse was located using the Doppler probe and the cuff then inflated until the pulse was obliterated. The cuff was then deflated slowly and the pressure noted when the pulse detected by the Doppler probe re-appeared. ABPI was calculated for each leg as the ratio of the higher of the two systolic pressures at the ankle and the average of the left and right brachial systolic pressures, unless there was a discrepancy ≥10mmHg in blood pressure values between the 2 arms, in which case the higher side systolic pressure was used. To standardise the blood pressure measurements all recordings were performed by myself, who had previously been trained in the measurement of ABPI by a consultant vascular surgeon.

A reduced ABPI in symptomatic patients confirms the existence of haemodynamically significant occlusive disease between the heart and ankle, with a lower ABPI indicating a greater haemodynamic severity of occlusive disease. ABPI values ≤0.9 in one or both legs were considered diagnostic of PAD. The leg with the lowest ABPI was used in subsequent analyses. As brachial and ankle systolic blood pressures are intrinsically variable

measurements, multiple measurements were taken avoid the risk of misclassification of a subject with PAD, based on ABPI <0.9. The absence of PAD was defined as levels from 0.91 – 1.39 in the absence of re-vascularisation of the lower limbs. In some patients with diabetes, renal insufficiency, or other diseases that cause vascular calcification, the tibial vessels at the ankle become non-compressible. This leads to a false elevation of the ankle pressure. These patients typically have an ABPI >1.40 and, in some of these patients, the Doppler signal at the ankle cannot be obliterated even at cuff pressures of 300 mmHg. ABPI values ≥1.4 were therefore excluded from the analysis.

The reproducibility of ABPI varies in the literature but it is significant enough that reporting standards require a change in ABPI of 0.15 in an isolated measurement for it to be considered clinically relevant, or >0.1 if associated with a change in clinical status [Norgren et al. 2007].

2.4.1.7 Intermittent Claudication Assessment

Subjects eligible for participation in the validation of the Edinburgh Claudication Questionnaire in South Asian languages, or in English, completed this in their chosen language either independently or with the aid of an interpreter reading the questionnaire as written.

The presence of intermittent claudication (IC) was defined by the criteria of the Edinburgh Claudication Questionnaire (ECQ) (Figure 2.4) [Leng et al. 1992]. Translated versions of this questionnaire were used in the chosen language of the patient if this was not English: Urdu, Punjabi, Hindi Gujurati and Bengali. The translated versions were validated as part of this research (Chapter 3.1). PAD was considered asymptomatic when ABPI <0.9 and the ECQ showed no IC. It was considered to be symptomatic if the ECQ suggested definite or atypical claudication.

Figure 2.4: Edinburgh Claudication Questionnaire [Leng et al. 1992]

(1) Do you get a pain or discomfort in your leg(s) when you walk? Yes □ No □

If you answered "Yes" to question (1), please answer the following questions. Otherwise you need not continue

(2) Does this pain ever begin when you are standing still or sitting? Yes □ No □

(3) Do you get it if you walk uphill or hurry? Yes □ No □

(4) Do you get it if you walk at an ordinary pace on the level? Yes □ No □

(5) What happens to it if you stand still?

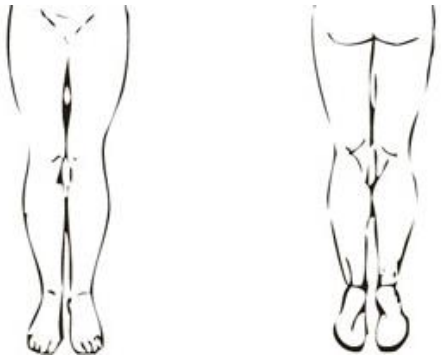
a. usually continues for more than 10 minutes □

b. usually disappears in 10 minutes or less □

(6) Where do you get this pain or discomfort?

Mark the place (s) with an 'X' on the diagrams below

Front Back



Definition of positive classification requires all of the following responses: "Yes" to (1), "No" to (2), "Yes" to (3), grade 1 "No" to (4), grade 2 "Yes" to (4). If these criteria are fulfilled, a definite claudicant is one who indicates the pain is in the calf, regardless of whether pain is also marked at other sites; a diagnosis of atypical claudication is made if the pain is marked in the thigh or buttock, in the absence of any calf pain. Subjects should not be considered to have claudication if pain is indicated in the hamstrings, feet, shins, joints or appears to radiate in the absence of any pain in the calf.

2.4.1.8 Blood sampling

If consent was granted, a random blood sample was obtained by atraumatic venepuncture and stored at 4°C for up to 4 hours before transportation to the central laboratory for storage at -70°C for batch analysis. Initial analyses include renal function and lipids using routine automated methodology using reagents from Roche Diagnostics within a clinical Biochemistry laboratory (Lewes, UK). Similarly, HbA1c was measured using semi-automated HPLC methodology (Menarini, Berkshire, UK). In addition, a full blood count was tested.

2.4.1.9 Enzyme-Linked Immunosorbant Assay (ELISA)

Blood was stored at 4°C for up to 4 hours before transportation to the central laboratory. It was then spun down at 1500 x g; the supernatant was then frozen at -70ºC for future batch analysis. Samples were tested for haemostatic cardiovascular risk factors using an established automated immunoassay technique using commercially available assays: VWF (Dako, Ely, UK), IL-6 (R&D systems reagents, Abingdon, UK), CRP (Biokit, SA, Barcelona, Spain), soluble P-selectin (R&D systems, Abingdon, UK). Intra-assay and inter-assay variances of all assays were <5% and <10% respectively. Lower limits of detection were IL-6: 9.375 pg/ml, sP-sel: 125 pg/ml, VWF: 65 iu/dl, CRP: 0.71 mg/l.

I personally assisted with the preparation of the supernatant and also assisted with the ELISAs for the haemostatic indices. After a period of training, I plotted all of the results on graphs using standard controls to obtain the values used in subsequent analyses.

2.4.1.10 Statistical Analysis

Statistical analysis was carried out using Minitab version 15 (State Coll, PA, USA). Data were summarised using mean, median, standard deviation and inter-quartile range for continuous parameters. The statistical analysis in this thesis followed standard statistics as a whole using univariate and multivariate analysis. These analyses will be addressed in the subsequent results chapters. The univariate analyses for differences in continuous variables for 2 groups are Student's t-test and Mann-Whitney U test and Chi-squared test for categorical data. Paired t-test was used to compare the values in either side of the neck and either leg. In cases where less than 5 participants were used in analysis Fisher's exact test was used. Spearman rank correlation coefficient was calculated to test the association between the CCIMT and a number of risk factors. Moving data forwards from univariate analyses into multivariate analyses automatically adjusts for multiple significance testing, i.e. a Bonferroni correction. Univariate analyses for 3 groups using ANOVA or Krusskal-Wallis is effectively a t-test or Mann-Whitney U test with a Bonferroni correction.

In forming models of analyses I've referred to a textbook by Altman (1991). In discussing the need for multiple significance testing (e.g. Bonferroni correction) in this cross-sectional, community based study I have read the papers by Bland & Altman, Rothman, Michels & Rosner and Greenland and Robins [Bland & Altman 1995, Rothman 1990, Michels & Rosner 1996, Greenland & Robins 1991].

Rothman stated, "Adjustments for multiple comparisons shields some observed associations from more intensive scrutiny by labelling them as chance findings, thereby defeating the

purpose of scientists" [Rothman 1990]. The increase in type 2 error accompanying adjustments for multiple comparisons dismisses potentially important associations by reducing sensitivity [Rothman 1990]. Michels and Robins state, "Cross-sectional studies are used to find undiscovered associations" and suggest that all associations could be studied and comparisons specified. By doing this further hypotheses will be generated for further investigation [Michels & Robins 1996]. They further state, "By adjusting for further comparisons and raising the significance level to unreachable heights would make every sensible investigation impossible" and conclude with, "The key to the sensible handling of data is careful interpretation of observed associations rather than artificial erection of barriers" [Michels & Robins 1996]. They use the Framingham study and the National Health and Nutrition Examination Survey (NHANES) as examples of large epidemiological studies which did not adjust for multiple significance testing as it was not feasible; results of which have been well published in peer reviewed journals. Finally, Bland & Altman suggest that when the data are not independent, as they are all on the same subjects using variables which may not be independent, the Bonferroni method is inappropriate as it will be highly conservative and may miss real differences [Bland & Altman 1995]. Following my reading of the above papers and consulting with Dr Blann, statistician in the Thrombosis, Haemostasis and Vascular Biology Unit in the University of Birmingham Department of Clinical Medicine, a p-value <0.05 was deemed to be significant in this thesis.

2.4.2 Hospital Based Study

2.4.2.1 Aim of hospital based study

The aim of this study was to investigate the plasma expression of markers of angiogenesis in 3 ethnic groups (South Asians, Blacks and White Europeans) in healthy volunteers, people with cardiovascular risk factors with no overt disease and in people with established cardiovascular disease. Furthermore the aim was to see if the plasma expression of angiogenic markers differs between these ethnic groups and differs across a spectrum of health and disease.

2.4.2.2 Participant Recruitment

Between October 2008 and September 2009 subjects were recruited into this study from 3 ethnic groups: South Asians (People originating from India, Pakistan and Bangladesh), Blacks (People originating from the Caribbean and sub-Saharan Africa) and Whites (White Europeans). Eligibility criteria are documented below.

Participants with symptomatic peripheral arterial disease (TIA or PAD) were recruited from Sandwell & West Birmingham Hospitals NHS Trust Department of Vascular Surgery. All had radiologically confirmed disease and either attended intermittent claudication clinics or attended for open surgery for peripheral arterial disease (Femoral-popliteal bypass, femoral-distal bypass, femoral endarterectomy, aorto-femoral bypass, axillo-femoral bypass, femoral-

femoral crossover, major limb amputation or carotid endarterectomy). Subjects having endovascular procedures were not included.

Risk factor controls were recruited from Sandwell & West Birmingham Hospitals NHS Trust Department of Medicine from patients attending cardiology and hypertension clinics. These participants had at least one cardiovascular risk factor (Hypertensive, Diabetic, Hypercholesterolaemia, Smokers) but not symptomatic PAD, excluded by Ankle Brachial Pressure Index (ABPI) >1.

Healthy volunteers were recruited from relatives of patients attending outpatient clinics or for elective general surgery at Sandwell & West Birmingham Hospitals NHS Trust and also from subjects participating in the E-ECHOES Study (See chapter 2.4.1.2 on page 82). Healthy volunteers were screened for cardiovascular risk factors and PAD, by medical history, medications history, and brachial blood pressure measurement and by measuring ABPI after 5 minutes in the supine position (Same technique mentioned above). Healthy volunteers were included if they had ABPI >1 and no cardiovascular risk factors.

Exclusion criteria for all subjects were infectious diseases, rheumatoid arthritis and other chronic inflammatory disorders, sepsis, malignancy, haemodynamically significant valvular heart disease, atrial fibrillation, renal failure, immunity-modulating drugs and hormone replacement therapy, recurrent venous thromboembolism, congestive cardiac failure, multiple sclerosis and anaemia.

The study was approved by Birmingham East, North & Solihull Local Research Ethnics Committee (08H1/20639) and written informed consent was granted from all study participants.

2.4.2.3 Clinical Assessment

Demographic data and medical history were collected using a standard interviewer-led questionnaire. Subjects were defined as having hypertension if they were previously known to have hypertension from medical records, using anti-hypertensive medication, or whose mean of 3 blood pressure recordings after 5 minutes rest was greater than 140/90. Similarly, subjects were defined as having diabetes mellitus if, their medical records stated it or if they were using anti-diabetic medication. Former smokers are those who have previously smoked tobacco but stopped >1 year prior to assessment. Smokers who stopped <1year prior to assessment will be defined as current smokers

All measurements (height, weight, body mass index, systolic and diastolic blood pressure and resting pulse) in study participants were undertaken by the student.

2.4.2.4 Blood Sampling

All subjects having vascular surgical procedures had venous blood drawn from the antecubital fossa within 24 hours prior to surgery by myself. Blood was stored on ice for a maximum of 2 hours before being spun down at 1500 x g; the supernatant was then frozen at -70ºC for future batch analysis. Samples were tested for angiogenic markers using commercially available immunoassays: (R&D systems Europe LTD Duosets): Angiopoietin-1, Angiopoietin-2, sTie2 receptor, Angiogenin. Intra-assay and inter-assay variances of all assays were <5% and <10% respectively. Lower limits of detection were 3.79ng/ml for Ang-1, 0.12ng/ml Ang-2, 0.09ng/ml Angiogenin and 1ng/ml sTie-2.

I personally assisted clinical scientists at Sandwell & West Birmingham NHS Trust in the preparation of ELISAs. After a period of training I then plotted all of the results on graphs using standard controls to obtain the values used in subsequent analyses.

2.4.2.5 Statistical Analysis

Data were summarised using mean, median, standard deviation (SD) and inter-quartile range [IQR] for continuous parameters, as appropriate. Student's t-test or the Mann-Whitney U test was used for differences in continuous variables and Chi-squared test and Fisher's exact test for categorical data. One way ANOVA was used to assess whether there were any differences between the 3 ethnic groups as a whole for continuous variables and Kruskal–Wallis test was used for non-parametric data, with Tukey's post-hoc analysis performed for inter-group differences. Section 2.4.1.10 (page 96) explains the rationale for not adjusting for multiple significance testing and as such, a p-value <0.05 was deemed to be significant. Statistical analyses were carried out using Minitab version 15 (State Coll, PA, USA).

CHAPTER 3:

SUB-STUDY TO ETHNIC ECHOCARDIOGRAPHIC HEART OF ENGLAND SCREENING (E-ECHOES) STUDY

3.1 Validation of the Edinburgh Claudication Questionnaire in 1st generation Black African-Caribbean and South Asian UK migrants

3.1.1 Abstract

Objectives: To determine the diagnostic accuracy of the Edinburgh Claudication Questionnaire (ECQ) in diagnosing intermittent claudication (IC) in 1st generation Black African-Caribbean UK migrants. To determine the diagnostic accuracy of translated versions of the ECQ in diagnosing IC in 1st generation South Asian UK migrants

Methods: Subjects were recruited from the Ethnic-Echocardiographic Heart of England Screening (E-ECHOES) study; a community based screening survey for heart failure in minority ethnic groups. Translated versions of the ECQ were prepared following a recognised protocol. All participants attending screening between October 2007 and February 2009 were asked to complete the ECQ in the language of their choice (English, Punjabi, Bengali, Urdu, Hindi or Gujarati). Subjects answering 'yes' to experiencing leg pain or discomfort on walking (ECQ question one) were asked to return to have Ankle Brachial Pressure Index (ABPI) measured within 2 weeks of initial assessment. For the purposes of this study an ABPI<0.9 in either leg was diagnostic for PAD and an ABPI<0.9 in combination with ECQ answers suggestive of IC (ECQ positive) was diagnostic for IC.

Results: 154 out of 2831 subjects participating in E-ECHOES (5.4%) were eligible to participate in this sub-study as they reported leg pain on exertion ('yes' to question one of the ECQ), for which 74.3% returned for ABPI measurement within 2 weeks of initial assessment. Non-returners were younger than subjects returning for ABPI measurement (59[9] vs. 65[11] years; $p = 0.015$). Punjabi, English and Bengali questionnaires identified participants with IC, so these versions of the ECQ were assessed in this study. The sensitivities (SN), specificities (SP), positive (PPV) and negative (NPV) predictive values were calculated for each version of the ECQ. English: SN: 50%; SP: 68%; PPV: 43%; NPV: 74%. Punjabi: SN: 50%; SP: 87%; PPV: 43%; NPV: 90%. Bengali: SN: 33%; SP: 50%; PPV: 13%; NPV: 73%. There were significant differences in diagnostic accuracy between the 3 versions (Punjabi: 83.8%; Bengali: 45%; English: 62.2%; $p < 0.0001$). No significant differences were found in sensitivity and specificity between illiterate and literate participants in any of the questionnaires and there was no significant difference in sensitivity and specificity between those under and over 60 years of age completing the ECQ.

Conclusions: This chapter's findings suggest that the ECQ is not as sensitive or specific a diagnostic tool for diagnosing IC in 1st generation Black African-Caribbean and South Asian UK migrants than in the Edinburgh Artery Study. This reflects the findings of other diagnostic questionnaires in these minority ethnic groups. Whether this is due to linguistic differences in describing pain or due to a lack of physical activity to bring on symptoms of IC or due to a difference in methodology in diagnosing PAD is not known.

3.1.2 Introduction

Peripheral arterial disease (PAD) is an important healthcare problem in developed nations and is associated with considerable morbidity and mortality. IC is the most common symptomatic manifestation of PAD, and typically occurs in up to one third of patients with this disease [Norgren et al. 2007]. IC is characterised by pain, aching or cramping in the calf, buttock, hip or thigh on ambulation that resolves upon rest. Symptoms arise from an inadequate blood supply to the peripheral arteries of the legs that result in anaerobic metabolism and build-up of lactic acid within the muscles. Only about a quarter of patients with IC will ever significantly deteriorate [Norgren et al. 2007].

Ankle brachial pressure index (ABPI) is the gold standard for the assessment of both asymptomatic and symptomatic PAD [Hirsch et al. 2006]. A value of <0.9 is indicative of PAD, with sensitivity and specificity of 95% for detecting angiogram positive disease [Dormandy et al. 2000; Hummel et al. 1978; Matzke et al. 2003]. Intermittent claudication can be diagnosed with the use of a questionnaire along with evidence of PAD. The Edinburgh Claudication Questionnaire (ECQ) was first validated by Leng et al. 1992 after noting that the previous WHO/Rose questionnaire had low sensitivity [Leng et al. 1992]. This patient administered questionnaire was administered to a predominantly European population and was found to be 91.3% sensitive and 99.3% specific for IC in comparison to a doctor made diagnosis [Leng et al. 1992]. Whilst there is always a potential for spectrum bias affecting the performance of a diagnostic text, the ECQ has been found to have excellent reliability and reproducibility after repeating the questionnaire at 6 months (Leng et al. 1992). The ECQ has been validated in French and Brazilian Portuguese [Lacroix et al. 2002; Aboyans et al. 2000; Makdisse et al. 2007] and in English in a community based study

in the Netherlands [Bendermacher et al. 2006] though not amongst languages of the Indian Sub-continent or amongst Black Caribbeans.

Studies have shown that questionnaires designed to diagnose cardiovascular diseases in European populations may not always be applicable in an ethnically diverse population [Fischbacher et al. 2001; Sorlie et al. 1996]. In order to meet the healthcare needs of the diverse populations which exists in the UK [Gill et al. 2007] it is important to know if any differences exist in the reporting of symptoms of disease and also whether current diagnostic tools designed in European populations are applicable in the diagnosis of disease in other ethnic groups.

The purpose of this study was to determine the diagnostic accuracy of the ECQ to diagnose IC, defined by ABPI <0.9 and ECQ answers suggestive of IC, in languages of the Indian sub-continent and also in English speaking Black African-Caribbean groups. As such only subjects reporting leg pain i.e. symptomatic disease were investigated in this chapter. Asymptomatic PAD is investigated elsewhere in this thesis.

3.1.3 Hypothesis to be tested

Translated versions of the Edinburgh Claudication Questionnaire in the following languages: Urdu, Bengali, Hindi, Punjabi and Gujarati will have equivalent sensitivity and specificity to that found in the Edinburgh Artery Study.

3.1.4 Patients & Methods

Translation of Edinburgh Claudication of Questionnaire

A diagrammatic representation of the translation process for each South Asian language is shown in figure 3.1. The original ECQ was formally translated into each of Hindi, Punjabi, Gujurati, Urdu and Bengali. For each translation a consortium comprising 3 bilingual healthcare professionals and a lay person was used to assess grammatical and semantic equivalence. A general consensus was made between these 4 people as to whether amendments needed to be made to the original translation. If so, the suggested amendments were sent back to the initial independent translator and a new version was produced. Once a translation was deemed to be acceptable, it was then independently back-translated into English. The back-translated version was then compared to the original English version of the ECQ. All translations were found to be grammatically and semantically equivalent. Translated versions of the ECQ are shown in Figure 3.2.

Figure 3.1: Schematic illustrating Edinburgh Claudication Questionnaire translation sequence

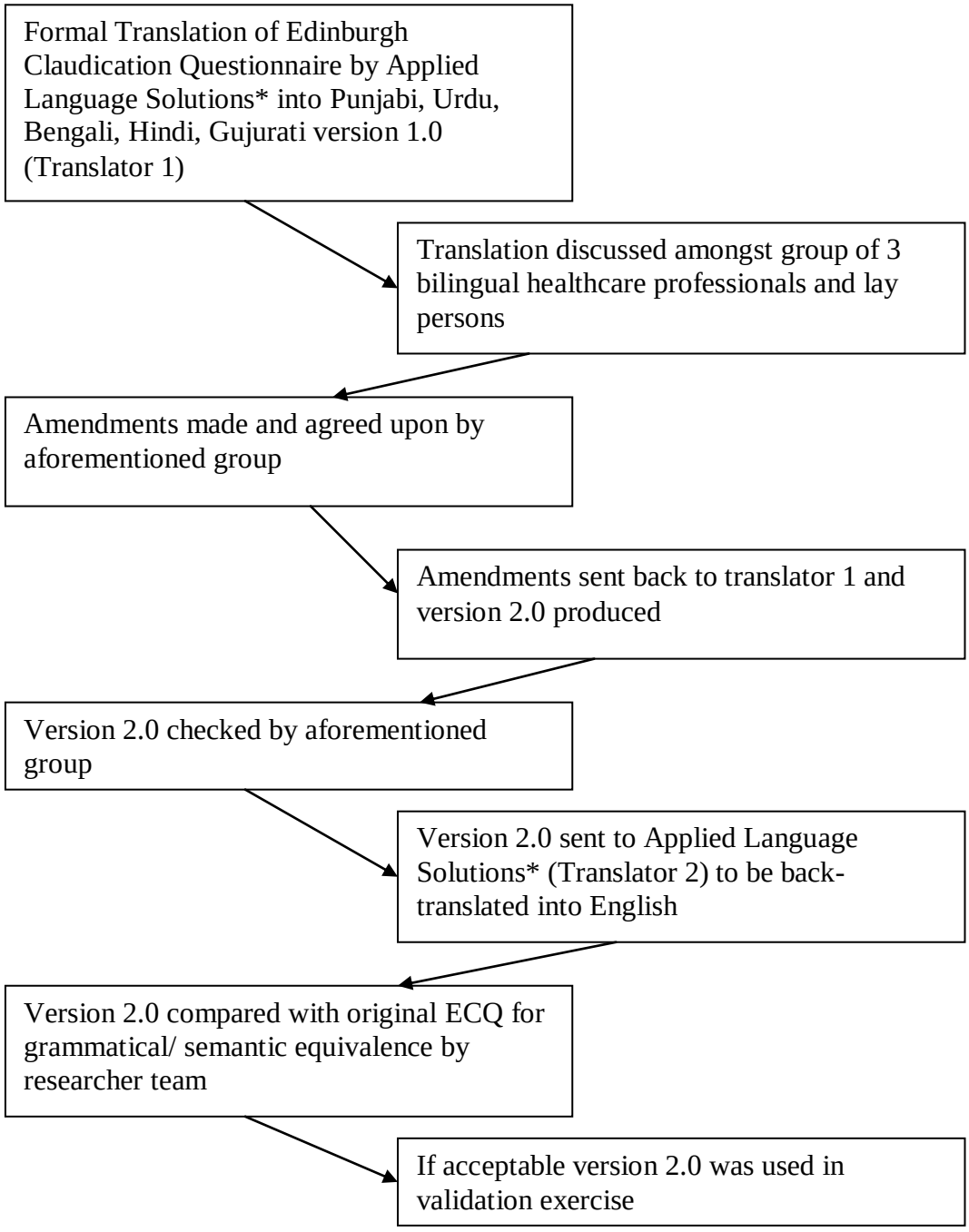


Figure 3.2: Edinburgh Claudication Questionnaire (ECQ) and Translations (Leng et al. 1992)

3.2.1 Original English Version of ECQ

(1) Do you get a pain or discomfort in your leg(s) when you walk? Yes □ No □

If you answered "Yes" to question (1), please answer the following questions. Otherwise you need not continue

(2) Does this pain ever begin when you are standing still or sitting? Yes □ No □

(3) Do you get it if you walk uphill or hurry? Yes □ No □

(4) Do you get it if you walk at an ordinary pace on the level? Yes □ No □

(5) What happens to it if you stand still?

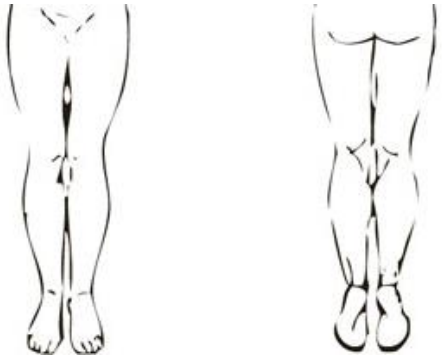
c. usually continues for more than 10 minutes □

d. usually disappears in 10 minutes or less □

(6) Where do you get this pain or discomfort?

Mark the place (s) with an 'X' on the diagrams below

Front Back



Definition of positive classification requires all of the following responses: "Yes" to (1), "No" to (2), "Yes" to (3), grade 1 "No" to (4), grade 2 "Yes" to (4). If these criteria are fulfilled, a definite claudicant is one who indicates the pain is in the calf, regardless of whether pain is also marked at other sites; a diagnosis of atypical claudication is made if the pain is marked in the thigh or buttock, in the absence of any calf pain. Subjects should not be considered to have claudication if pain is indicated in the hamstrings, feet, shins, joints or appears to radiate in the absence of any pain in the calf.

3.2.2 ECQ- Bengali Translation

এডিনবরা পায়ের ব্যথার (ক্লডিকেশন) প্রশ্নমালা:

1. যখন আপনি হাঁটেন তখন কি আপনার পায়ে কোন ব্যথা বা অস্বস্তি হয়?
   হ্যাঁ ☐
   না ☐
   আমি হাঁটতে পারছি না

যদি আপনি প্রশ্ন 1-এ ''হ্যাঁ'' উত্তর দিয়ে থাকেন অনুগ্রহ করে নিম্নলিখিত প্রশ্নগুলির উত্তর দিন। তা না হলে আপনাকে আর উত্তর দিতে হবে না।

2. যখন আপনি স্থির হয়ে দাঁড়িয়ে বা বসে থাকেন তখন এই ব্যথা কি কখনো শুরু হয়?
   হ্যাঁ ☐
   না ☐

3. চড়াই পথে হাঁটলে বা তাড়াহুড়ো করলে আপনার কি তা হয়?
   হ্যাঁ ☐
   না ☐

4. আপনি যখন সাধারণ গতিতে সমতল দিয়ে হাঁটেন তখন কি আপনার ব্যথা হয়?
   হ্যাঁ ☐
   না ☐

5. স্থির হয়ে দাঁড়িয়ে থাকলে ব্যথাটির কি হয়?
   সাধারণত 10 মিনিটের বেশী সময় ধরে তা থাকে ☐
   সাধারণত 10 মিনিট বা তার কম সময়ে তা মিলিয়ে যায় ☐

6. আপনার কোথায় এই ব্যথা বা অস্বস্তি হয়?
   নিচের চিত্রটিতে জায়গাগুলিতে ''X'' দিয়ে দাগ দিন

সামনে

পিছনে

3.2.3 ECQ- Gujurati Translation

ધી એડિનબર્ગ કલાઉડીકેશન પ્રશ્નોત્તરીઃ

1. જ્યારે તમે ચાલો છો ત્યારે તમને પગમાં દુઃખાવો કે તકલીફ થાય છે?
   હા
   ના
   હું ચાલી શકતો નથી.

જો પ્રશ્ન 1 નો જવાબ 'હા' હોય તો કૃપા કરી નીચેના પ્રશ્નોના જવાબ આપો.
અન્યથા તમારે ચાલુ રાખવાની આવશ્યકતા નથી.

2. તમે સ્થિર ઊભા રહો કે બેસો ત્યારે હંમેશા દુઃખાવો શરૂ થાય છે?
   હા
   ના

3. જ્યારે તમે ટેકરી પર કે ઝડપથી ચાલતા હો ત્યારે તમને દુઃખાવો થાય છે?
   હા
   ના

4. જ્યારે તમે મુસાફરીમાં સામાન્ય રીતે ચાલતા હો ત્યારે તમને દુઃખાવો થાય છે?
   હા
   ના

5. જો તમે સ્થિર ઊભા રહો ત્યારે શું થાય છે?
   સામાન્ય રીતે10 મીનિટ કે તેથી વધુ સમય સુધી ચાલુ રહે છે
   સામાન્ય રીતે10 મીનિટ કે ઓછા સમયમાં ગાયબ થઈ જાય છે

6. આ દુઃખાવો કે તકલીફ તમને કયાંથી થાય છે?
   નીચે આપેલ નકશા પર "X" ની નિશાની મૂકો.

આગળ

પીઠ

3.2.4 ECQ - Hindi Translation

एडिनबर्ग पंगुता (क्लॉडिकेशन) प्रश्नावली:

1. जब आप चलते/चलती हैं, तो क्या आपके पैर/पैरों में दर्द या तकलीफ होती है?
   हाँ □
   नहीं □
   मैं चल नहीं पाता/पाती हूँ

यदि आपने प्रश्न 1 का उत्तर "हाँ" दिया हो, तो कृपया निम्नलिखित प्रश्नों के उत्तर दें। अन्यथा आप आगे के प्रश्नों को छोड़ सकते/सकती हैं।

2. क्या यह दर्द कभी-कभी तब भी शुरू होता है जब कि आप एक जगह खड़े/खड़ी हों अथवा बैठे/बैठी हों?
   हाँ □
   नहीं □

3. क्या आपको यह दर्द तब होता है जब कि आप पहाड़ी/चढ़ाव चढ़ रहे/रही हों या तेज़ चाल चल रहे/रही हों?
   हाँ □
   नहीं □

4. क्या आपको यह दर्द तब होता है जब कि आप सामान्य चाल से समतल सतह पर चल रहे/रही हों?
   हाँ □
   नहीं □

5. जब आप स्थिर खड़े/खड़ी हो जाएँ, तो इस दर्द का क्या होता है?
   सामान्यतः 10 मिनट से अधिक समय तक बना रहता है □
   सामान्यतः 10 मिनट या उससे कम समय में चला जाता है □

6. यह दर्द या तकलीफ आपको कहाँ महसूस होती है?
   नीचे के चित्र में उन स्थानों पर "x" का चिन्ह लगाएँ

आगे

पीछे

3.2.5 ECQ- Punjabi Translation

ਲੰਗੜਾਉਣ ਬਾਰੇ ਐਡਿਨਬਰਾ ਸਵਾਲਨਾਮਾ:

1. ਕੀ ਤੁਹਾਨੂੰ ਤੁਰਦੇ ਹੋਏ ਆਪਣੀ(ਆਂ) ਲੱਤ(ਤਾਂ) 'ਚ ਦਰਦ ਜਾਂ ਤਕਲੀਫ਼ ਹੁੰਦੀ ਹੈ?
   ਹਾਂ □
   ਨਹੀਂ □
   ਮੈਂ ਤੁਰ ਨਹੀਂ ਸਕਦਾ/ਸਕਦੀ

ਜੇਕਰ ਸਵਾਲ 1 ਦਾ ਜਵਾਬ ਤੁਸੀਂ ''ਹਾਂ'' ਦਿੱਤਾ ਸੀ ਤਾਂ ਕਿਰਪਾ ਕਰਕੇ ਇਨ੍ਹਾਂ ਸਵਾਲਾਂ ਦੇ ਜਵਾਬ ਦਿਓ| ਨਹੀਂ ਤਾਂ ਤੁਹਾਨੂੰ ਅੱਗੇ ਵੱਧਣ ਦੀ ਲੋੜ ਨਹੀਂ ਹੈ|

2. ਕੀ ਕਦੇ ਇਹ ਦਰਦ ਤੁਹਾਡੇ ਸਿੱਧੇ ਖੜ੍ਹੇ ਰਹਿਣ ਜਾਂ ਬੈਠਣ 'ਤੇ ਸ਼ੁਰੂ ਹੁੰਦਾ ਹੈ?
   ਹਾਂ □
   ਨਹੀਂ □
3. ਕੀ ਤੁਹਾਨੂੰ ਕੋਈ ਚੜ੍ਹਾਈ ਚੜ੍ਹਨ ਜਾਂ ਛੇਤੀ ਤੁਰਨ 'ਤੇ ਇਹ ਹੁੰਦਾ ਹੈ?
   ਹਾਂ □
   ਨਹੀਂ □

4. ਕੀ ਤੁਹਾਨੂੰ ਕਿਸੇ ਸਮਤਲ ਥਾਂ 'ਤੇ ਸਾਧਾਰਨ ਗਤੀ ਨਾਲ ਤੁਰਨ 'ਤੇ ਇਹ ਹੁੰਦਾ ਹੈ?
   ਹਾਂ □
   ਨਹੀਂ □

5. ਤੁਹਾਡੇ ਸਿੱਧੇ ਖੜੇ ਰਹਿਣ 'ਤੇ ਇਸਦਾ ਕੀ ਹੁੰਦਾ ਹੈ?
   ਆਮ ਤੌਰ 'ਤੇ 10 ਮਿੰਟਾਂ ਤੋਂ ਵੱਧ ਜਾਰੀ ਰਹਿੰਦਾ ਹੈ □
   ਆਮ ਤੌਰ 'ਤੇ 10 ਮਿੰਟਾਂ ਜਾਂ ਘੱਟ ਸਮੇਂ ਦੇ ਅੰਦਰ ਦੂਰ ਹੋ ਜਾਂਦਾ ਹੈ □

6. ਤੁਹਾਨੂੰ ਇਹ ਦਰਦ ਜਾਂ ਤਕਲੀਫ਼ ਕਿੱਥੇ ਹੁੰਦੀ ਹੈ?
   ਹੇਠ ਦਿੱਤੇ ਗਏ ਚਿੱਤਰ 'ਚ "x" ਦੇ ਚਿੰਨ੍ਹ ਵਾਲੀਆਂ ਥਾਵਾਂ 'ਤੇ ਨਿਸ਼ਾਨ ਲਗਾਓ

ਅਗਲਾ ਪਾਸਾ ਪਿਛਲਾ ਪਾਸਾ


3.2.6 ECQ- Urdu Translation

ایڈنبرا عروج سوالنامہ

1. کیا آپ اپنی ٹانگ (ٹانگوں) میں چلتے وقت درد یا بے چینی محسوس کرتے ہیں ؟
   ہاں
   نہیں
   میں چلنے کے قابل نہیں ہوں

اگر آپ نے سوال نمبر 1 کا جواب " ہاں " میں دیا ہے تو برائے مہربانی باقی سوالات کے جوابات بھی دیں.
دوسری صورت میں آپ کو جاری رکھنے کی ضرورت نہیں ہے.

2. کیا یہ تکلیف اس وقت شروع ہوتی ہے جب آپ ساکت کھڑے یا بیٹھے ہوتے ہیں؟
   ہاں
   نہیں

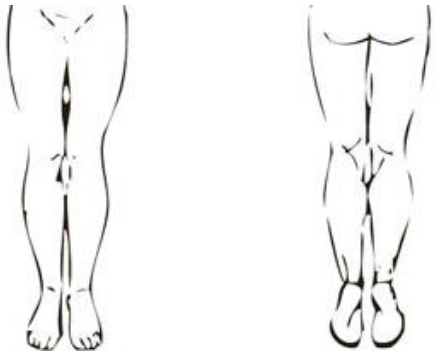
3. کیا یہ تکلیف آپ کو چڑھائی چڑھتے ہوئے یا تیز چلتے ہوئے محسوس ہوتی ہے؟
   ہاں
   نہیں

4. کیا یہ تکلیف اس وقت محسوس ہوتی ہے جب آپ ہموار سطح پر معمول کی رفتار سے چل رہے ہوں؟
   ہاں
   نہیں

5. اگر آپ کھڑے ہوتے ہیں تو کیا ہوتا ہے؟
   10 منٹ سے زیادہ جاری رہتی ہے
   عام طور پر 10 منٹ یا اس سے کم عرصے میں ختم ہو جاتی ہے

6. آپ کو یہ بے چینی یا درد کہاں محسوس ہوتا ہے؟
   نیچے دی گئی شکل پر " X " کا نشان لگائیں

سامنے پیچھے



Study Design &Recruitment

This was a sub-study of the E-ECHOES UK community survey screening South Asian and Black African-Caribbean residents of Birmingham aged 45years or over for heart failure, described in detail in Chapter 2.4.1.2 on page 83.

All subjects screened between October 2007 and February 2009 were asked to complete the ECQ in their chosen language. Subjects reporting leg pain or discomfort on walking (answering 'yes' to question one of ECQ) were subsequently invited back for ABPI assessment within 2 weeks of their initial assessment. The study was approved by the local research and ethics committee and written informed consent was obtained from all patients. Figure 3.3 is a diagrammatic representation of the recruitment process.

Questionnaire Validation

Patients attending for assessment as part of E-ECHOES were asked to complete the ECQ in their preferred language, including English (Figure 3.3). All literate patients completed the questionnaire independently and illiterate patients were provided with a bilingual interpreter if required and the questions were read out to them as written. Participants responding negatively to question one of the ECQ ("Do you get a pain or discomfort in your leg(s) when you walk?") in any language were not included in the analysis.

PAD Assessment

A description of how PAD was assessed is found in section 2.4.1.6 on page 92.

Results of the Edinburgh Claudication Questionnaire

The diagnosis of a positive questionnaire was made on the basis of the original guidelines – see figure 3.2. The respondent must have answered *yes* to question 1, *no* to question 2, *yes* to question 3, *usually disappears in less than 10 minutes* to question 5 and in question 6, mark the calf, thigh or buttock regions. A negative questionnaire was one that did not have this exact combination. Question 4 was only used to define the severity of claudication if present.

Definition of Intermittent Claudication

A positive questionnaire along with an ABPI <0.9 would be diagnostic of intermittent claudication for the purpose of this study.

Statistical analysis

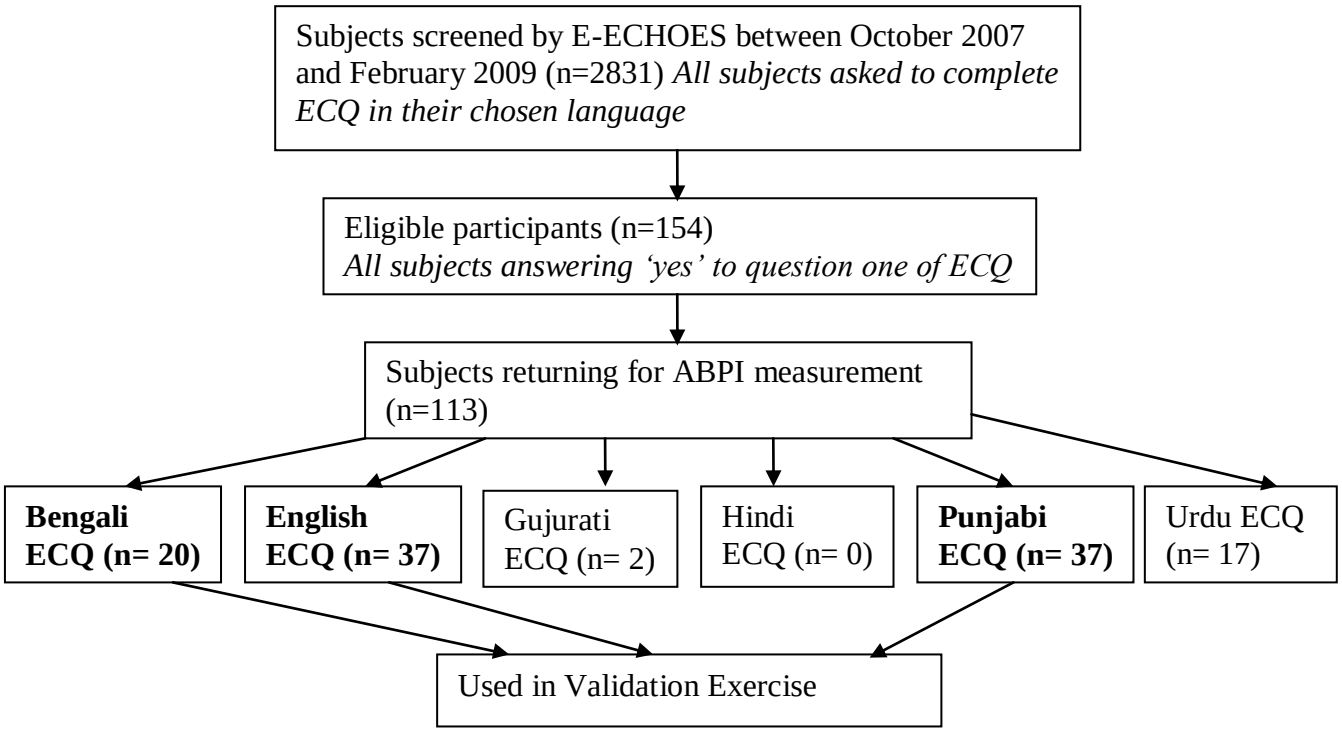
Questionnaire performance was assessed using Minitab 15 (State Coll, PA, USA). The sensitivity, specificity, positive predictive value, negative predictive values were calculated. Diagnostic accuracy was then calculated by dividing the number of individuals under correct classification on the ECQ by the total number of subjects assessed. Data with a continuous variation were subjected to the Anderson-Darling test to determine mode of distribution. If normally distributed, such data is summarised using mean and standard deviation, and if non-normally distributed by median and inter-quartile range. One way ANOVA was used to assess whether there were any differences in continuous variables between the 3 groups (speakers of English, Punjabi or Bangladeshi). Fisher's exact test was used in 2x2 tables between participants with ABPI <0.9 and participants without for each question of the ECQ to determine whether any particular question was responsible for contributing to the

differences in overall sensitivity and specificity of the study questionnaires. Significance was defined as $p<0.05$.

3.1.5 Results

All subjects (n=2831) screened as part of E-ECHOES between October 2007 and February 2009 were asked to complete the ECQ in their chosen language. 154 participants (5.4%) answering 'yes' to question one "Do you get pain or discomfort in your leg(s) when you walk?" were invited to attend for a subsequent assessment of ABPI of which, 74.3% of eligible participants attended (Figure 3.3).

Figure 3.3: Diagrammatic representation of recruitment process



Demographic data for attendees and non-returners is shown in table 3.17. Non-returners were significantly younger (59 standard deviation [SD][9] vs. 65[11] years; p=0.015) and had significantly more Gujurati speakers (14.3 vs. 1.8%; p=0.003). There were no differences in cardiovascular risk factors and illiteracy rate between those participating in validation exercise and those not.

Table 3.1: Characteristics of subjects eligible for participation in validation study

Variable	ABPI attendees (n=113) (SD)	Non-returners (n=41) (SD)	p-value
Age	65(11)	59 (9)	0.015
Male (%)	53	52	0.916
Illiteracy (%)	29.5	31	0.875
Language			
-Bengali (%)	17.7	10.7	0.371
-English (%)	32.7	46	0.175
-Guajarati (%)	1.8	14.3	0.003
-Punjabi (%)	32.7	25	0.429
-Urdu (%)	15	3.6	0.088
mean SBP (mmHg)	138 (25)	135 (16)	0.62
mean DBP (mmHg)	77 (12)	79 (8)	0.585
BMI	29 (5)	31 (5)	0.06
Ever Smoker (%)	56.6	58.6	0.846
Current Smoker (%)	15.3	31	0.057
Hypertensive (%)	65.5	69	0.732
Diabetic (%)	50	34.5	0.145
CAD (%)	35.5	25	0.301
CBVD (%)	16.1	13.8	0.762

SD: Standard deviation; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; CAD: coronary artery disease; CBVD: cerebrovascular disease

The total number of respondents completing each version of the ECQ is illustrated in figure 3.3. All participants completing the ECQ in English were 1st generation Black Caribbeans. All participants completing the translated versions were 1st generation South Asian migrants. Of the 6 languages, only English, Punjabi and Bengali had participants with ABPI <0.9 and ECQ suggestive of claudication, so these were used in the questionnaire validation in this pilot study.

The mean age of participants in this study was 65 years of whom 53% were males and 29.5% were illiterate. PAD was evident in 21.2% [95% CI: 14.7-29.7] of subjects whom gave a positive response to ECQ question one and subsequently invited back for ABPI assessment. Intermittent claudication was present in 8.8% [95% CI: 4.9-15.5] of these participants. There was no significant age difference between those with PAD and those without. The participant demographics of those completing each version of the ECQ are shown in table 3.2.

Table 3.2: Demographic details of subjects by language of Edinburgh Claudication Questionnaire

Variable	English* (SD) [IQR] n=37	Punjabi (SD)[IQR] n=37	Bengali (SD) [IQR] n=20	p-value
Age (years)	67 **(**9)	66 (13)	64 (9)	0.577
% Male	55.9	41.7	66.7	0.192
% Illiteracy	0	45.9	50	<0.0001
Age of leaving school	16 [15-17]	15 [9-17]	11 [8-15]	0.648
% Higher education	2.7	2.7	5	0.874
% ABPI <0.9	32.4	16.6	20	0.237
% IC	16.2	8.1	5.0	0.345
ABPI	0.95	1.03	0.98	0.155
Diabetes mellitus (%)	65.4	34.5	58.8	0.062
Hypertension (%)	73.9	60	70.6	0.532
Ever Smoker (%)	61.1	40.5	57.9	0.182
Current Smoker (%)	14.8	20.6	10.5	0.616
Coronary artery disease (%)	37	28.1	52.6	0.216
Cerebrovascular disease (%)	24.1	9.4	11.8	0.251
Body Mass Index	30.4 (5.5)	28.2 (5.4)	28.2 (4.3)	0.271
Waist circumference (cm)	92.6 (14.8)	94 (13.9)	97.4 (11.5)	0.488

SD: standard deviation; ABPI: ankle brachial pressure index; IC: intermittent claudication; * 1st Generation Black Caribbean UK migrants. Data presented as mean (SD) or percentage. Illiteracy: people who never attended school in any country

Punjabi Questionnaire:

37 Subjects completed this translated version (Table 3.3). Their mean age was 66 (3) years and 41.7% were male. 49.5% were illiterate and required a bilingual translator to read and interpret the subjects' responses (Table 3.2). For subjects attending school, the median age of leaving education was 15 [inter-quartile range (IQR) 9-17] years, with only 2.7% attending higher education (Table 3.2). The sensitivity of the Punjabi ECQ was 50%, specificity 87%, positive predictive value (PPV) 43% and negative predictive value 90% (Table 3.4). The diagnostic accuracy of this version was 83.8%. This study attempted to look for differences in sensitivity and specificity between illiterate (sensitivity 33.3%; specificity 92.9%) and literate (sensitivity 66.6%; specificity 86.7%) subjects. There were no differences in diagnostic accuracy between these 2 groups (84.2 vs.83.3%). I also attempted to investigate whether age would affect the sensitivity and specificity of the ECQ and found subjects under the age of 60 years had higher sensitivity (100 vs. 50%) but lower specificity (83.3 vs.93%) than those older than 60 years. However due to very small numbers of true claudicants significance was not reached. No differences in diagnostic accuracy between those under or over 60 years were reported (<60 years: 84.6%; >60 years: 84.2%).

Bengali Questionnaire:

20 Subjects completed the Bengali translation (Table 3.3), in which the mean age was 64 (9) years and 66.7% were male (Table 3.2). 50% were illiterate, and required a bilingual translator to complete the questionnaire, and of those attending school the median age of leaving was 11 [IQR 8-15] years (Table 3.2). Only 5% attended higher education. The sensitivity and specificity of this translation were 33.3% and 50% respectively and PPV and NPV were 11% and 73% respectively (Table 3.4). The diagnostic accuracy of the Bengali

ECQ was 45%. None of the illiterate Bengali speakers had ABPI <0.9 and so this study could not compare the sensitivity of this questionnaire between those attending school and those whom never attended. The specificity between the former and latter was 66.7 and 50% respectively but this failed to reach statistical significance. Likewise no Bengali subjects below the age of 60 had ABPI<0.9 and no significant difference in sensitivity was found with age. Diagnostic accuracy did not differ significantly between the 2 groups (<60years: 50%; >60 years: 58.3%).

English Questionnaire:

37 Subjects completed the original English version of the ECQ, all of whom were African Caribbean. Their mean age was 67 (9) years and 55.9% were male (Table 3.2). All subjects attended school and the median age of leaving school was 16 [IQR 15-17] years (Table 3.2). 2.7% attended higher education. Sensitivity and specificity were 50 % and 68% respectively and PPV and NPV were 43 % and 74% (Table 3.4). The diagnostic accuracy of this version was 62.2%. The sensitivity of the ECQ in those under 60 and over 60 years of age were 50 vs. 55.6% respectively and specificity were 100 vs. 75% respectively but results were not significant due to the low number of subjects. The diagnostic accuracy was 66.7% for <60years and 56.7% >60 years of age.

Table 3.3: 2x2 Tables illustrating the ECQ results and ABPI measurements

	ABPI <0.9	**ABPI >0.9**
ECQ positive	A	B
ECQ negative	C	D

ECQ positive + ABPI<0.9 (a) = Intermittent Claudication
Sensitivity = a / (a+c)
Specificity = d / (b+d)
Positive predictive value = a / (a+b)
Negative predictive value = d / (c+d)
Diagnostic accuracy = (a+d)/ (a+b+c+d)

3.3.1 English (n=37)

	ABPI <0.9	**ABPI >0.9**
ECQ positive	6	8
ECQ negative	6	17

3.3.2 Punjabi (n=37)

	ABPI <0.9	**ABPI >0.9**
ECQ positive	3	4
ECQ negative	3	27

3.3.3 Bengali (n=20)

	ABPI <0.9	**ABPI >0.9**
ECQ positive	1	8
ECQ negative	3	8

Table 3.4: Sensitivity, Specificity, Positive and Negative predictive values of the Edinburgh Claudication Questionnaire

Language	Sensitivity(%) [95% CI]	Specificity(%) [95% CI]	PPV(%) [95% CI]	NPV(%) [95% CI]
English*	50 [22-78]	68 [50-86]	43 [16-68]	74 [55-91]
Punjabi	50 [10-90]	87 [75-99]	43 [5-79]	90 [79-100]
Bangladeshi	33.3 [0-86]	50 [26-75]	13 [0-31]	73 [45-99]

* 1st Generation Black Caribbean UK migrants; PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval

Overall Cohort:

There was no difference in age, sex distribution and prevalence of cardiovascular risk factors between the English, Punjabi and Bengali ECQ groups. There were also no differences in body mass index and waist circumference in these 3 groups. There were significant differences in illiteracy between South Asian participants and African Caribbean subjects (Table 3.2). However there was no statistically significant difference in the sensitivity and specificity of the ECQ between illiterate and literate subjects. Of those attending school no statistically significant differences were found in median age of leaving and proportion of people attending higher education.

This study investigated the sensitivity and specificity of each of the questions 2 to 6 in the ECQ (Table 3.5). In all languages question 3, "Do you get it [Pain] when you walk uphill or hurry?" was the most sensitive. The least sensitive question was question 5, pertaining to duration of pain. This question was overall the most specific in the diagnosis of intermittent claudication.

Table 3.5: Sensitivity, Specificity, Positive and Negative predictive values of each question of the Edinburgh Claudication Questionnaire

3.5.1: English (n=37)

ECQ Question	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Q2	25	44	8	75
Q3	100	20	19	100
Q4	75	44	20	90
Q5	41.7	48	13	81
Q6	66.7	20	14	76

3.5.2: Punjabi (n=37)

ECQ Question	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Q2	50	45.2	7	91
Q3	100	32.3	8	100
Q4	100	32.3	11	100
Q5	66.7	64.5	14	96
Q6	83.3	22.6	9	94

3.5.3: Bengali (n=20)

ECQ Question	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Q2	75	62.5	10	98
Q3	100	62.5	5	100
Q4	75	40	6	97
Q5	25	50	3	93
Q6	100	62.5	5	100

3.1.6 Discussion

This study has shown that translated versions of the ECQ into South Asian languages and the original English version in 1st generation Black Caribbean migrants have lower sensitivity and specificity than the original version [Leng et al. 1992] but similar levels reported in other populations [Lacroix et al. 2002; Bendermacher et al. 2006]. This study also reports significant differences in diagnostic accuracy between the Punjabi, Bengali and English versions. This study differs from the study by Leng et al. in that I used ABPI, an objective measure of PAD, defined by a value<0.9, rather than a doctor made diagnosis of PAD based on history taking alone.

The ECQ was developed and validated as part of the Edinburgh Artery Study [Leng et al. 1992] with the objective of improving the sensitivity of the WHO/Rose Claudication Questionnaire [Rose 1962]. The researchers questioned 300 participants over the age of 55 with leg pain and reported 91.3% sensitivity and 99.3% specificity of the ECQ at diagnosing intermittent claudication. The original ECQ was used in large observational study investigating people presenting to their general practitioner with symptoms suggestive of IC, in the Netherlands, which reported a much lower sensitivity of 56.2% [Bendermacher et al. 2006]. Makdisse et al. recently published a Brazilian Portuguese version of the ECQ and reported 85% sensitivity and 93% specificity [Makdisse et al. 2007]. Previously Aboyans et al. published a French version with 86.5% sensitivity and 95.6% specificity [Aboyans et al. 2000] which was subsequently repeated by Lacroix et al., who reported a marked difference in the questionnaire's sensitivity at 47% [Lacroix et al. 2002].

Studies have previously shown that questionnaires designed to diagnose cardiovascular disease in White European populations may not always be applicable in an ethnically diverse population [Fischenbacher et al. 2001; Sorlie et al. 1996; Raczynski et al. 1993]. The preliminary findings of this study suggest the ECQ is not a sensitive or specific diagnostic tool for IC in Black African-Caribbean and South Asian groups. This reflects the findings of other researchers using the Rose Angina questionnaire [Fischenbacher et al. 2001; Sorlie et al. 1996]. It is possible that South Asians and African Caribbeans may describe pain differently than white European populations, which may account for the apparent differences in sensitivity and specificity when compared to Leng et al. [Leng et al. 1992]. Indeed it has previously been reported that the Rose Angina questionnaire has a lower sensitivity and specificity in South Asians than in white Europeans [Fischenbacher et al. 2001]; site of pain and duration of pain being least likely to score a positive response to Rose Angina questionnaire in both South Asian men and women. This study found question 5 of the ECQ, pertaining to duration of pain to have the least sensitivity of all of the questions in the ECQ in all versions, which may partly explain the low sensitivity and specificity found overall. People of African descent have also been reported to be less likely to score positively to angina using the Rose questionnaire and less likely to seek treatment than white group [Raczynski et al. 1993].

This study used an objective measure (ABPI) to diagnose PAD rather than clinical assessment only, and its findings of lower sensitivity and specificity, positive and negative predictive values of the ECQ when compared to Leng et al. are comparable to other population surveys [Lacroix et al. 2002; Bendermacher et al. 2006]. The Edinburgh artery study used clinical assessment (i.e. patient history), rather than ABPI, to diagnose PAD and

as such there would be an increased chance of matching ECQ results with the presence of PAD as both diagnoses would have focussed on history of symptomatic disease. Using an objective measure such as ABPI would put patients with ABPI<0.9 but ECQ negative as being false positives and patients representing symptoms of IC (ECQ positive) but ABPI>0.9 would be classed as false negatives, which would lower the sensitivity and specificity and overall accuracy of the questionnaire in its current form.

Limitations

The main limitation of this study was recruitment of participants answering 'yes' to question one of the ECQ and subsequent re-attendance for ABPI measurement. As such the results found in this study lack statistical power and so firm conclusions regarding the sensitivity and specificity of the ECQ in the groups studied can't be made. Previous studies used more symptomatic participants in their validation exercises [Makdisse et al. 2007; Bendermacher et al. 2006]. However, as the E-ECHOES study [Gill et al. 2009] was based within primary care and screened all eligible subjects whether or not they were symptomatic with cardiovascular disease; only 5.4% of the 2831 E-ECHOES subjects were eligible to take part in this sub-study. It was not possible to measure ABPI on all 2831 subjects and I acknowledge I may have missed people with asymptomatic PAD. However, the purpose of this study was to validate a diagnostic tool to detect symptomatic PAD, in the form of intermittent claudication, rather than detect asymptomatic PAD and so not measuring ABPI on the remainder of the 2831 subjects who did not report leg pain on exertion shouldn't have affected its results. The attempt to validate the ECQ in several languages meant the number of expected cases of IC in each language questionnaire was going to be low. Of the eligible participants, only 74.3% returned for ABPI assessment. This may have resulted in responder

bias and may have affected the validity of the ECQ. Potential differences in participant characteristics between non-returners and those participating in the validation exercise were analysed and suggest that the significantly younger age of the former group may have resulted in work-related commitments preventing a return visit for ABPI measurement. However, the younger age group might also have had a lower risk of PAD and therefore may not have yielded more cases of IC. It is possible the inclusion of illiterate participants may have contributed to the low sensitivity, specificity and diagnostic accuracy of the ECQ versions. However, this study found no significant differences in these groups. It is also possible that the age of participants may have contributed to the diagnostic accuracy of the questionnaires. However this study found no significant differences. This study's findings must be interpreted with caution however due to aforementioned sample size limitations.

Another limitation is that ABPI alone was used as a diagnostic tool for PAD, rather than confirming the diagnosis with imaging. As such, subjects with arterial calcification may have had falsely elevated ABPI, contributing to some of the false negative results, which may have masked an underlying diagnosis of claudication. Other diseases potentially affecting our population, such as Takayasu disease, would not have been detected by ABPI alone and as such may have also contributed to the false negatives that were found in this study.

Another limitation of this study is that for intermittent claudication to manifest, patients must exercise sufficiently to experience pain. Various surveys have reported much lower levels of physical activity amongst British South Asians than in the general population, especially among women and older people [Hayes et al. 2006, Fischbacher et al. 2004]. There is a lack of literature on levels of physical activity amongst Black Caribbean UK migrants [Health

Survey for England 2004]. The lack of sufficient physical activity amongst study participants to produce symptoms from PAD might explain the high level of false negatives found in this study.

3.1.7 Conclusion

This study's findings suggest the original English ECQ is not a sensitive or specific tool in the diagnosis of intermittent claudication in UK Black African Caribbean migrants. Punjabi and Bengali versions also did not show high sensitivity and specificity when compared to the Edinburgh Artery Study. The implications of this are that medical professionals should be cautious when applying healthcare questionnaires designed in one population group to other groups because of the risk of spectrum bias. The low sensitivity this study reported means that subjects with a questionnaire not indicative of IC can't be ruled out as having IC and the low specificity means that subjects with a questionnaire suggestive of IC can't be ruled in as having this condition. The low sensitivity and specificity may thus over of under-represent the true extent of IC in the subjects investigated in this study. Furthermore, questionnaires that rely upon physical activity to produce symptoms, such as the ECQ, should be avoided in populations known to have low levels of physical activity as they are likely to miss the true extent of symptomatic disease. Larger studies, involving minority ethnic groups, need to be performed before firm conclusions can be made about the utility of the ECQ in non-White groups.

The high concordance between cardiovascular risk factors and leg pain reported in this sub-study should prompt clinicians to perform an objective assessment of PAD, such as ABPI, in suspicious patients presenting to primary and secondary care independent of whether they fulfil the definition of IC based on the ECQ. Subjects with ABPI <0.9 should undergo further investigations to confirm the diagnosis and if appropriate, should be commenced on best medical therapy.

3.2 The contribution of Cardiovascular Risk Factors to Peripheral Arterial Disease in South Asians and Blacks

3.2.1 Abstract

Objectives: To determine whether differences exist in prevalence of peripheral arterial disease (PAD) between South Asians (people originating from India, Pakistan and Bangladesh) and Blacks (Black Caribbean and Black African), the two largest minority ethnic groups in the UK. To determine if associations with cardiovascular risk factors and PAD differ between these two ethnic groups.

Methods: 572 (356 South Asian and 216 Black) subjects ≥45 years took part in this sub-study to a community screening project, the Ethnic-Echocardiographic Heart of England Screening (E-ECHOES) study. All subjects completed an interviewer-led questionnaire, anthropometric measurements and blood sampling. Ankle brachial pressure index (ABPI) was calculated and PAD was diagnosed by ABPI <0.9.

Results: The mean age was 62 years overall with no difference between the two ethnic groups. The prevalence of PAD was 13.2% [95% confidence interval (CI) 9.7–16.7] in South Asians and 10.2% (95% CI 6.2–14.2) in Blacks with no significant difference between the two ethnic groups. No statistically significant differences in ABPI were found between South Asians and Blacks overall and in men. However, the prevalence of PAD was higher (16.3 vs. 6.1%; p = 0.011) and ABPI lower (1.01 (0.12) vs. 1.04 (0.13); p=0.039) in South Asian women compared to Black women, which could not be explained by the prevalence of traditional cardiovascular risk factors. On multivariate analysis male sex (p=0.008), SBP (p=0.017), diabetes (p=0.022) and smoking (p=0.037) were all independent predictors of

PAD in South Asians. In Blacks only age (p=0.003) was an independent predictor of PAD, while a non-significant trend between smoking (P=0.056) and PAD was found.

Conclusion: The prevalence of PAD is similar in South Asians and Blacks, and similar to levels reported in pre-dominantly White populations. South Asian women appeared to have a higher prevalence of PAD than Black women, which is not explained by traditional cardiovascular risk factors. Further, greater powered, research is required in order to investigate possible socio-demographic or genetic reasons for this finding.

3.2.2 Introduction

Peripheral arterial disease (PAD) is an important healthcare problem in developed nations and is associated with considerable morbidity and mortality. PAD is the disease process resulting from obstruction of large peripheral arteries, exclusive of the coronary and intracranial cerebrovascular system, most commonly due to atherosclerosis [Norgren et al. 2007]. Most typically, it is referred to in relation to the lower limbs. PAD is an indicator of widespread atherosclerosis in other vascular territories, including the cerebral and coronary circulations [Norgren et al. 2007] and considerable overlap exists between them [CAPRIE steering committee 1996; Pasternak et al. 2004].

Several population based studies [Fowkes et al. 1991; Meijer et al. 1998; Diehm et al. 2004] based on predominantly white populations have found the prevalence of PAD to be between 6 to 18% over the age of 55. The prevalence rises with age and has been found to be approximately 20% in people over 70 years of age [Regensteiner et al. 2002] and up to 60% in the over 85 age group [Meijer et al. 1998]. However, little is known about the prevalence of PAD in non-White populations. The aim of this study was to estimate the prevalence of PAD in the two largest UK minority ethnic groups (South Asians and Blacks) and make associations with traditional cardiovascular risk factors. South Asians (People originating from India, Pakistan and Bangladesh) and Blacks (Black Caribbean and Black African) comprise 75% of minority ethnic groups within the UK [Gill et al. 2007; National Statistics Online]. In order to meet the healthcare needs of the diverse population which exists in the UK, it is important to know if any differences in disease epidemiology exist between different minority ethnic groups.

3.2.3 Hypotheses to be tested

There will be a difference in prevalence of PAD (Defined by Ankle Brachial Index (ABPI) <0.9) between South Asians and Blacks.

Ethnic differences will exist in associations between traditional cardiovascular risk factors (Smoking, diabetes mellitus, dyslipidaemia and hypertension) and PAD in South Asians and Blacks.

3.2.4 Patients and Methods

Study Design &Recruitment

Details of the study design and recruitment into the E-ECHOES study and into this sub-study are described in detail in Chapter 2.4.1.2 on page 82.

Clinical Assessment

Demographic data and medical history were collected using a standard questionnaire and recording form. Data were derived from both the subject and their medical records. All clinical assessments were performed at the same visit. Subjects were defined as having hypertension if they were previously known to have hypertension from medical records, using anti-hypertensive medication, or whose mean of 3 blood pressure recordings after 5 minutes rest was greater than 140/90. Similarly, subjects were defined as having diabetes mellitus if, their medical records stated it or if they were using anti-diabetic medication. Subjects were defined as being illiterate if they never attended school in any country. Former smokers are those who have previously smoked tobacco but stopped >1 year prior to assessment. Smokers who stopped <1year prior to assessment will be defined as current smokers.

PAD Assessment

A description of how PAD was assessed is found in section 2.4.1.6 on page 92.

Laboratory Assessment

If consent was granted, blood was drawn from the antecubital fossa using the Vacutainer system. Random blood was analysed for full blood count, urea and electrolytes, liver function tests, glucose, HbA1c and total cholesterol and triglycerides. Consent for blood sampling was obtained in 269 out of 572 (47%) of this sub-study to E-ECHOES (143 out of 356 (40.2%) South Asians and 126 out of 216 (58.3%) Blacks).

Statistical Analysis

Statistical analysis was undertaken using Minitab version 15 (State Coll, PA, USA). Data were summarised using mean, median, standard deviation and inter-quartile range for continuous parameters. Student's t-test and Mann-Whitney U test were used for differences in continuous variables and Chi-squared test for categorical data. Spearman rank correlation coefficient was calculated to test the association between the ABPI and a number of risk factors. Based on previous reports on the dangers of multiple significance testing in cross-sectional studies described in chapter 2.4.1.10, a Bonferroni correction was not performed and a p-value <0.05 was deemed significant. In order to test the hypothesis that ethnic differences will exist in associations between traditional cardiovascular risk factors and PAD in South Asians and Blacks, these were entered into multivariate analyses along with other variables significant on univariate analysis.

3.2.5 Results

Between March 2008 and February 2009, 574 subjects (358 South Asians and 216 Blacks) were recruited, aged between 45 and 100 years; 2 subjects with ABPI >1.39, were excluded, bringing the total analysed to 356 South Asians and 216 Blacks. The ethnic breakdown of the South Asian group was: 40.4% Pakistani, 38.9% Indian, 16.9% Bangladeshi and 2.8% East African Asian, which is broadly representative of the distribution of these ethnic groups within Birmingham, UK [Neighbourhood statistics].The South Asian group were analysed as one group rather than separately as Indian, Pakistani and Bangladeshi in a hope to improve the statistical power of any findings.

Table 3.6 shows clinical, demographic and ABPI characteristics of the 2 groups. The South Asian group had more first generation migrants, greater illiteracy, higher serum triglycerides and HbA1c and lower HDL-Cholesterol and less ever smokers than Blacks. In the whole sample, 69 subjects had PAD; 47 were South Asians whilst 22 were Black.

Table 3.6: Characteristics of study subjects by Ethnic Group

Variable	South Asian n=356 (SD)[IQR]	Blacks n=216 (SD) [IQR]	p-value
Age (years)	61 (11)	63 (11)	0.108
Male (%)	58.7	46.8	0.005
1st generation migrants (%)	98.6	86.1	<0.0001
Illiteracy (%)	30.1	0	<0.0001
Body Mass Index	28 (4.9)	29.5 (5.8)	0.001
Waist circumference (cm)	98.4 (13.1)	97.6 (12.8)	0.452
Mean SBP (mmHg)	142 (20)	145 (18)	0.085
Mean DBP (mmHg)	82 (11)	83 (10)	0.43
Pulse Pressure (mmHg)	60 (16)	62 (17)	0.137
Resting Pulse rate (bpm)	80 (13)	78 (13).	0.042
CAD (%)	12.9	6.5	0.01
CBVD (%)	3.7	4.8	0.5
Diabetes (%)	30.6	27.3	0.4
Hypertension (%)	70.8	75.5	0.224
Never Smoker (%)	71.9	51.9	<0.0001
Ex-Smoker (%)	16.6	27.3	0.002
Current Smoker (%)	11.5	20.8	0.003
Antiplatelet medication usage (%)	34.8	33.8	0.8
Cholesterol lowering medication (%)	48	42.6	0.2
Triglycerides (mmol/l)	2.0 [1.3-2.7] ¥	1.36 [0.7-2.2] ᴷ	<0.0001
LDL-C (mmol/l)	2.3 [1.8-2.9] ¥	2.52 [1.8-3.1] ᴷ	0.297
HDL-C (mmol/l)	1.1 [0.8-1.2] ¥	1.32 [1.1-1.6] ᴷ	<0.0001
Total Cholesterol (mmol/l)	4.4 [3.8-5.2] ¥	4.63 [3.7-5.3] ᴷ	0.295
HbA1c (%)	6.7 (1.3) ¥	6.1 [5.7-6.6]ᴷ	0.0007
lowest side ABPI	1.02 (0.1)	1.04 (0.1)	0.11
ABPI <0.9 (%)	13.2	10.2	0.283

SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin, ABPI: ankle brachial pressure index; BPM: beats per minute; CAD: coronary artery disease; CBVD: cerebrovascular disease; ¥: n=143; ᴷ: n=126; IQR: Inter-quartile range; SD: standard deviation

South Asians

In South Asians, the prevalence of PAD was 13.2% (95% CI: 9.7-16.7) overall, with no significant gender difference (16.3% in women (95% CI: 10.1-21.9) and 11% in men (95% CI: 6.8-15.2)). Table 3.7 illustrates univariate comparisons of the South Asian subjects by sex. Women were older on migration to the UK (23 vs. 22 years; p=0.04) and had a higher body mass index (BMI) than men (29.1 vs. 27.2; p<0.0001). The prevalence of illiteracy in the language of origin was high in both groups but statistically significantly higher in women (40.1% vs. 23%; p=0.001). Men were more likely to be hypertensive (76.1% vs. 63.3%; p=0.009), and diabetic (34.9% vs. 25.2%; p=0.05) with worse diabetic control (HbA1c 6.93% vs. 6.36%; p= 0.007) than women. Men were also more likely to have ever smoked (45.5% vs. 3.4%; p<0.0001) and be current smokers (18.7% vs. 1.4%; p<0.0001).

The higher proportion of men on antiplatelet (43.1% vs. 23.1%; p<0.0001) and cholesterol lowering medication (52.6% vs. 41.5%; p=0.038) may reflect the greater prevalence of cardiovascular risk factors in this group.

Table 3.7: Univariate Comparisons between male and female South Asian subjects

Variable	Male n=209 (SD) [IQR]	Female n=147 (SD) [IQR]	p-value
Age (years)	61 (11)	60 (11)	0.495
Illiteracy (%)	23	40.1	0.001
Body Mass Index	27.2	29.1	<0.0001
Waist circumference (cm)	97.9 (12.4)	99.2 (14)	0.374
Mean SBP (mmHg)	144 (20)	140 (20)	0.106
Mean DBP (mmHg)	84 (11)	80 (11)	0.005
Pulse Pressure (mmHg)	60 (16)	60 (16)	0.898
Resting Pulse rate (bpm)	80 (13)	80 (13)	0.877
CAD (%)	15.3	9.5	0.109
CBVD (%)	5.3	3.4	0.404
Diabetes (%)	34.9	25.2	0.05
Hypertension (%)	76.1	63.3	0.009
Ever Smoker (%)	45.5	3.4	<0.0001
Current Smoker (%)	18.7	1.4	<0.0001
Antiplatelet medication usage (%)	43.1	23.1	<0.0001
Cholesterol lowering medication (%)	52.6	41.5	0.038
Triglycerides (mmol/l)	2.01 [1.28-2.81] ¥	1.93 [1.33-2.62] ᴋ	0.565
LDL-C (mmol/l)	2.44 [1.59-2.76] ¥	2.24 [2-3.11] ᴋ	0.09
HDL-C (mmol/l)	0.97 [0.77-1.14] ¥	1.14 [1.03-1.34] ᴋ	<0.0001
Total Cholesterol (mmol/l)	4.29 [3.54-4.95] ¥	4.56 [3.96-5.51] ᴋ	0.072
HbA1c (%)	6.93 (1.29) ¥	6.36 (1.11) ᴋ	0.007
ABPI	1.03 (0.14)	1.01 (0.12)	0.133
ABPI <0.9 (%)	11	16.3	0.144

SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin, ABPI: ankle brachial pressure index; CAD: coronary artery disease; CBVD: cerebrovascular disease; ¥: n= 89; ᴋ: n=52; IQR: Inter-quartile range; SD: standard deviation

Univariate comparisons between PAD and non-PAD groups in South Asian subjects are shown in Table 3.8. On univariate analysis, **s**ubjects with PAD were older than those without, were more likely to be male, had lower ABPI and had higher SBP and PP.

Table 3.8: Univariate comparisons between PAD and non-PAD groups in South Asian subjects

Variable	PAD n=47 (SD) [IQR]	Non-PAD n=309 (SD) [IQR]	p-value
Age (years)	64 (11)	60 (11)	0.039
Male (%)	70.2	34.6	<0.0001
lowest side ABPI	0.8 (0.1)	1.06 (0.11)	<0.0001
BMI	28.2 (3.7)	27.9 (5)	0.142
mean SBP (mmHg)	150 (21)	141 (19.9)	0.005
Pulse Pressure (mmHg)	69 (18)	58 (16)	0.001
Hypertensive (%)	74.5	70.2	0.55
Diabetic (%)	21.3	32.4	0.125
CAD (%)	8.5	13.2	0.36
CBVD (%)	4.3	4.9	0.858
Total Cholesterol (mmol/l)	4.26 [3.2-5.2]¥	4.37 [3.8-5.2] ᴷ	0.363
LDL-C (mmol/l)	2.13 [1.4-2.8]¥	2.31 [1.8-2.9] ᴷ	0.303
HDL-C (mmol/l)	0.9 [0.7-1.4]¥	1.06 [0.9-1.2] ᴷ	0.410
Triglycerides (mmol/l)	2.1 [0.9-3.2]¥	1.99 [1.3-2.7] ᴷ	0.838
HbA1c (%)	6.9 [6.2-9.2]¥	6.3 [6.0-7.0] ᴷ	0.107
Ever smoker (%)	21.3	29.1	0.265
Illiteracy (%)	21.3	31.4	0.157

SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin, ABPI: ankle brachial pressure index; CAD: coronary artery disease; CBVD: cerebrovascular disease; IQR: Inter-quartile range; SD: standard deviation; ¥: n=14; ᴷ: n=129

Table 3.9 illustrates possible predictors of PAD in South Asians on binary logistic regression. Using PAD as the response, adjusting for traditional cardiovascular risk factors the following variables were entered into multivariate analysis: Age, sex, SBP, diabetes and smoking history. Due to the low level of blood sampling, cholesterol (TC, LDL-C or HDL-C) was not adjusted for or entered into the multivariate model. With the categorical variables the OR reflects the log transformed odds of a subject with, for example, diabetes having PAD. For continuous variables (SBP and age) the dataset was standardised around the mean value and standard deviation. The OR in this circumstance is the log transformed odds of having PAD with every unit rise (e.g. every unit rise in mmHg or year of age) in the variable.

In the multivariate model in table 3.9 male sex, SBP, diabetes and smoking were all independent predictors of PAD in South Asians.

Table 3.9: Possible Predictors of PAD on multivariate analysis in South Asians

Variable	Odds Ratio	95% CI	p-value
Male sex	2.81	1.31-6.04	0.008
SBP	1.02	1.001-1.04	0.017
Diabetes mellitus	2.14	1.12-4.12	0.022
Smoking	1.72	1.03-2.87	0.037
Age	1.02	0.99-1.05	0.115

Table 3.10 investigates possible independent predictors of ABPI on linear regression analysis. Traditional cardiovascular risk factors were adjusted for. The following variables were included in the model: age, sex, SBP, diabetes and smoking. Due to the low level of blood sampling, cholesterol (TC, LDL-C or HDL-C) was not adjusted for or entered into the model.

Of the variables entered into the multivariate model in table 3.10, SBP, male sex, age and smoking were independent predictors of ABPI.

Table 3.10: Possible predicators of ABPI on linear regression in South Asian subjects

Variable	p-value
SBP	<0.0001
Male sex	0.005
Age	0.03
Smoking	0.043
Diabetes mellitus	0.095

Blacks

In Blacks, the prevalence of PAD was 10.2% overall (95%CI: 6.2-14.2) and was statistically significantly higher in men (14.9% (95%CI: 8.04-21.7) vs. 6.1% (95%CI: 1.7-10.3); p=0.034). Table 3.11 illustrates univariate comparisons between male and female Black subjects.

Women had higher BMI than men (31 vs. 27.7; p<0.0001) and a larger waist circumference (99.2 vs. 95.8cm; p=0.049). Whilst no difference was found in total cholesterol, LDL-C and triglycerides, women had higher HDL-C (p=0.034). Men were more likely to ever had smoked (68.3% vs. 30.4%; p<0.0001) and be current smokers (31.7% vs. 11.3%; p<0.0001).

There was no difference in the prevalence of hypertension and diabetes and no difference in the proportion of each sex taking antiplatelets and cholesterol lowering medication. All Black subjects attended school.

Table 3.11: Univariate comparisons between male and female Black Subjects

Variable	Male (n=101) (SD) [IQR]	Female (n=115) (SD) [IQR]	p-value
Age (years)	64 (11)	62 (11)	0.212
Illiteracy (%)	0	0	*
Body Mass Index	31 (6.3)	27.7 (4.6)	<0.0001
Waist circumference (cm)	95.8 (11.7)	99.2 (13.5)	0.049
Mean SBP (mmHg)	147 (19)	142 (18)	0.086
Mean DBP (mmHg)	83 (10)	83 (10)	0.743
Pulse Pressure (mmHg)	64 (17)	60 (17)	0.093
Resting Pulse rate (bpm)	75 (10)	80 (15)	0.002
CAD (%)	5.9	7.8	0.587
CBVD (%)	4	4.3	0.887
Diabetes (%)	30.7	29	0.748
Hypertension (%)	79.2	72.2	0.231
Antiplatelet medication usage (%)	38.6	29.6	0.161
Ever Smoker (%)	68.3	30.4	<0.0001
Current Smoker (%)	31.7	11.3	<0.0001
Cholesterol lowering medication (%)	47.5	38.3	0.170
Triglycerides (mmol/l)	1.2 [0.92-2.43] ¥	1.43 [0.7-2.05] ᴷ	0.784
LDL-C (mmol/l)	2.22 [1.6-2.94] ¥	2.57 [1.89-3.13] ᴷ	0.098
HDL-C (mmol/l)	1.24 [0.87-1.5] ¥	1.37 [1.13-1.69] ᴷ	0.034
Total Cholesterol (mmol/l)	4.48 [3.54-5.2] ¥	4.69 [3.86-5.42] ᴷ	0.107
HbA1c (%)	6.52 (1.24) ¥	6.37 (1.59) ᴷ	0.529
lowest side ABPI	1.04 (0.15)	1.04 (0.13)	0.844
ABPI <0.9 (%)	14.9	6.1	0.034

SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin, ABPI: ankle brachial pressure index; CAD: coronary artery disease; CBVD: cerebrovascular disease; ¥: n=54; ᴷ: n=75; *: too small sample size for analysis; IQR: Inter-quartile range; SD: standard deviation

Table 3.12 illustrates univariate comparisons between PAD and non-PAD groups in Black subjects. Subjects with PAD were significantly older and had lower ABPI and lower BMI than those without PAD. Subjects with PAD were also more likely to be hypertensive and therefore had higher SBP and PP than those without.

Table 3.12: Univariate comparisons between PAD and non-PAD groups in Black subjects

Variable	PAD (n=22) (SD) [IQR]	Non-PAD (n=194) (SD) [IQR]	p-value
Age (years)	71 (12.4)	62 (11)	0.001
Male (%)	54.5	45.4	0.44
lowest side ABPI	0.75 (0.1)	1.07 (0.1)	<0.0001
BMI	28.7 (5)	29.6 (5.9)	0.029
mean SBP (mmHg)	155 (18)	144 (18)	0.012
Pulse Pressure (mmHg)	75 (20)	60 (16)	0.003
Hypertensive (%)	95.5	73.2	0.021
Diabetic (%)	31.8	26.8	0.617
CAD (%)	4.5	7.2	*
CBVD (%)	4.5	4.1	*
Total Cholesterol (mmol/L)	4.68 [3.9-5.4]¥	4.6 [3.7-5.3] ᴷ	0.769
LDL-C (mmol/l)	2.73 [2.1-3.4]¥	2.5 [1.8-3.1] ᴷ	0.836
HDL-C (mmol/l)	1.35 [0.9-1.7]¥	1.32 [1.1-1.6] ᴷ	0.808
Triglycerides (mmol/l)	1.4 [0.2-2]¥	1.36 [0.9-2.2] ᴷ	0.463
HbA1C (%)	6.24 [5.9-12.8]¥	6.1 [5.7-6.6] ᴷ	0.594
Ever smoker (%)	40.9	49	0.391

SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin, ABPI: ankle brachial pressure index; CAD: coronary artery disease; CBVD: cerebrovascular disease; IQR: Inter-quartile range; SD: standard deviation
¥: n=10; ᴷ: n=116; *: too small sample size for analysis

Table 3.13 illustrates possible independent predictors of PAD on binary logistic regression in Blacks. As with the South Asian group PAD was used as the response. Adjusting for traditional cardiovascular risk factors the following variables were entered into multivariate analysis: Age, sex, SBP, diabetes and smoking history. Due to the low level of blood sampling, cholesterol (TC, LDL-C or HDL-C) was not adjusted for or entered into the model. Of the variables entered into the multivariate model, Age was the only variable independently predictive of PAD.

Table 3.13: Possible Predictors of PAD on multivariate analysis in Blacks

Variable	Odds Ratio	95% CI	p-value
Age	1.08	1.03-1.14	0.003
Smoking	2.05	0.98-4.28	0.056
Diabetes mellitus	1.78	0.66-4.83	0.254
SBP	1.01	0.99-1.04	0.287
Male sex	0.65	0.21 – 2.02	0.457

Table 3.14 investigates possible independent predictors of ABPI on linear regression analysis. Traditional cardiovascular risk factors were adjusted for. The following variables were included in the model: age, sex, SBP, diabetes and smoking. Due to the low level of blood sampling, cholesterol (TC, LDL-C or HDL-C) was not adjusted for or entered into the model. SBP and smoking were the only variables that remained independent predictors of ABPI on linear regression analysis.

Table 3.14: Possible predictors of ABPI in Black subjects on linear regression

Variable	p-value
SBP	0.001
Smoking	0.031
Age	0.126
Male sex	0.249
Diabetes mellitus	0.709

Ethnic comparisons in PAD groups:

No statistically significant differences were found in the prevalence of PAD (ABPI <0.9) between South Asians and Blacks when both sexes were combined (Respectively 13.2 (9.7-16.7) % vs. 10.2 (6.2-14.2) %; p=0.283). Likewise there was no difference when comparing ABPI between the 2 ethnic groups (South Asian 1.04(0.14) vs. Black 1.02(0.13); p=0.110) (Figure 3.4). South Asian women had a greater prevalence of PAD (16.3% vs. 6.1%; p=0.011) than Black women and lower ABPI (1.01 (0.12) vs. 1.04 (1.13) respectively; p=0.039). No statistically significant difference in prevalence of PAD was found between South Asian and Black men (11% vs. 14% respectively; p=0.47). No statistically significant differences were found in ABPI either (South Asian 1.03 (0.14) vs. Black 1.04 (0.15); p=0.638).

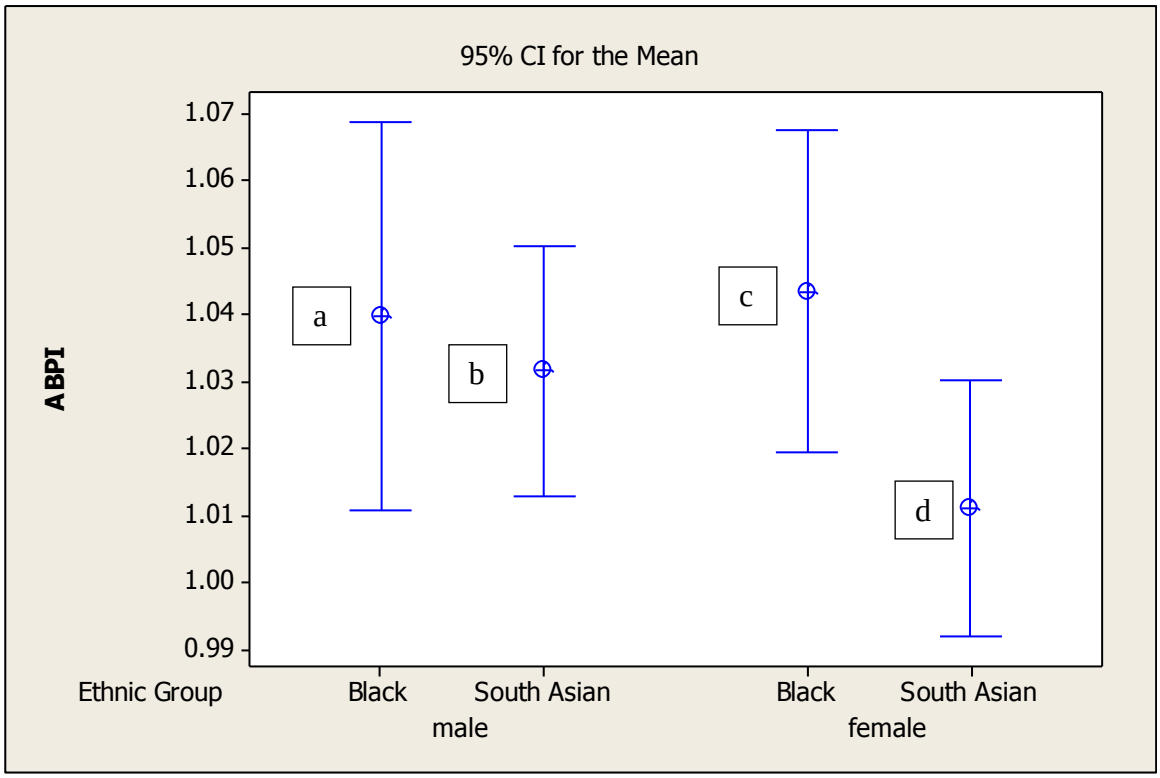
Overall Blacks with PAD were older than South Asians with this disease (71 vs. 64 years; p=0.028) and had more prevalent hypertension (95.5% vs. 74.9%; p=0.038) and South Asians had greater illiteracy (21.3 vs. 0%; p<0.0001). None of the other demographic variables were significantly different between the two PAD groups (Tables 3.8 & 3.12).

Amongst the female subjects the only statistically significant differences in prevalence of traditional cardiovascular risk factors was in the proportion of current (p=0.001) and ever smokers (p<0.0001) for which South Asian women reported a lower prevalence than Black women. South Asian women also had lower HDL-C (p=0.003) and higher triglycerides (p=0.018) than Blacks women. South Asian women also had much higher levels of illiteracy (p<0.0001).

Amongst the male subjects, no statistically significant differences were found in prevalence of hypertension and diabetes. However Blacks had a higher prevalence of current ($p<0.0001$) and ever ($p=0.011$) smokers and South Asian men had lower levels of HDL-C ($p=0.001$).

Figure 3.4 illustrates ABPI comparisons by sex and ethnic group. No statistically significant differences were found in ABPI between the 4 groups over all. However South Asian women have statistically significantly lower ABPI than Black women.

Figure 3.4: ABPI comparisons by sex and ethnic group



ABPI Values: a: 1.04 (0.15); b: 1.03 (0.14); c: 1.04 (0.13); d: 1.01 (0.12); p=0.115 on ANOVA. Difference between Black females (c) and South Asian females (d) p<0.05 on Tukey's post hoc analysis

3.2.6 Discussion

To date there is a paucity of data on PAD amongst minority ethnic groups, in particular amongst South Asians. This pilot study shows that the prevalence of PAD is similar amongst the South Asian and Black groups and indeed similar to previously published prevalence data in other populations [Premalatha et al. 2000, Criqui et al. 2005; UK Prospective Diabetes Study Group 1994; Mohan et al. 1995, Chaturvedi et al. 2007; Collins et al. 2005; McDermott et al. 2005], which supports the validity of this study.

This study reports findings similar to existing literature in that the rates of ever smokers and current smoking is lower in South Asians than in Blacks, overall and in each sex and that men are more likely to be smokers than women in both ethnic groups [HSE 2004]. This study observed that smoking was an independent predictor of ABPI and of PAD in South Asians, which differs from the findings of a previous Indian population study [Premalatha et al. 2000]. The data from this study also shows smoking to be an independent predictor of ABPI in Blacks but whilst there was a trend towards smoking being a significant predictor of PAD ($p=0.056$) in this group it failed to reach statistical significance, which may be due to the low numbers of subjects with PAD in this small cross-sectional study.

The prevalence of diabetes in this study was much higher in South Asians and in Blacks than in previous studies [Chaturvedi et al. 1994; Diabetes in the UK 2004]. In diabetic patients, the risk of PAD is increased by age, duration of DM and blood glucose control [Selvin et al. 2006; UK Prospective Diabetes Study Group 1998]. This study found that the OR of having PAD in the presence diabetes in South Asians was twofold higher than those with normal ABPI, which reflects results found in India [Premalatha et al. 2000]. The lack of an

independent association between diabetes and ABPI in South Asians could possibly be due to the fact that subjects with diabetes may have falsely elevated ABPI due to vascular calcification. Whilst the prevalence of diabetes in this study of predominantly 1st generation South Asian migrants was much higher than in those living in India, the mean age of participants in this study was also much older, which may partly explain this finding.

Amongst Blacks, this study failed to find statistically significant differences in the prevalence of diabetes in subjects with PAD on univariate analysis and no independent associations between diabetes and PAD or ABPI on multivariate analyses. This may either be because there is no association between these variables in this ethnic group; it might be because of falsely elevated ABPI amongst the diabetic subjects or might be due to the small numbers of participants in this study. This finding might also be because of the high prevalence of co-existent hypertension with diabetes which meant that any possible independent associations were lost. Further research is needed to investigate possible reasons for the findings of this study.

The most frequent dyslipidaemia associated with PAD are raised triglyceride levels and low HDL-cholesterol [Smith et al.2004]. In the literature, South Asians appear to have a less favourable lipid profile than other ethnic groups, with lower HDL-Cholesterol and higher triglycerides [Chaturvedi et al. 1994; Health Survey for England 1999, Miller et al. 1984; Li et al. 2006; Zoratti et al. 1998], which become more pronounced on migration from the Indian subcontinent [Bhatnagar et al. 1995; Patel et al. 2006]. On the other hand Blacks have a more favourable lipid profile [Miller et al. 1984; Allison et al. 2006; Whincup et al. 2002;

Lip et al. 2007]. This study reflects previous reports of the differences in lipid profile between ethnic groups.

Several components of blood pressure, including PP, SBP and diastolic blood pressure (DBP) have been shown to be independent cardiovascular risk factors. Hypertension is known to be a risk factor for both symptomatic and asymptomatic PAD [Murabito et al. 1997; Dieter et al. 2002]. Hypertension is well known to be more prevalent in Blacks, compared to South Asian and White ethnic groups [Agyemang et al. 2003] in the UK. The prevalence of hypertension in this study was very high in both ethnic groups. Previous (predominantly white population based) studies have shown that 35-55% of patients with PAD also had hypertension at presentation [Cheng et al. 1999; Vidi et al. 1996; Makin et al. 2001]. However, US studies have shown that among patients with PAD, the prevalence of hypertension was higher in African Americans than in Whites, and lower in Asians [Collins et al. 2003; Criqui et al.2005]. This study found Blacks with PAD had a significantly higher prevalence of hypertension than South Asians with PAD.

This study found no significant differences in the prevalence of hypertension between the PAD and normal ABPI groups in South Asians on univariate analysis but when blood pressure was analysed as a continuous variable, both SBP and PP were higher in the PAD group. On multivariate analysis, SBP was an independent predictor of both PAD and ABPI suggesting this variable might be important for a reduced ABPI and subsequent development of PAD. However causal associations cannot be made in this cross-sectional study.

In Blacks a difference was found in the prevalence of hypertension between the PAD and normal ABPI groups on univariate analysis. Subjects with PAD also had higher SBP and PP than those with normal ABPI. On multivariate analysis while SBP didn't remain an independent predictor of PAD on logistic regression, possibly due to the low number of subjects with PAD, when ABPI was analysed as a continuous variable SBP was found to be the most statistically significantly associated independent predictor of ABPI on linear regression. This suggests that even in the presence of other cardiovascular risk factors, SBP could be very important in the development of a reduced ABPI and therefore subsequent PAD. However, as with the South Asian group, causal associations cannot be made from this cross-sectional study.

Another important finding of this study is that the prevalence of coronary artery disease (CAD) and cerebrovascular disease (CBVD) was low in the study subjects. As expected South Asians had trend towards more prevalent CAD than Blacks but there was no difference in CBVD. The prevalence and presence of co-existent CAD and CBVD with PAD was very low and not different to the non-PAD group in both ethnic groups on univariate analysis. While PAD has been reported as conferring a greater risk of developing CAD and CBVD in predominantly white populations [Golomb et al. 2006; Criqui et al. 1998, Dormandy et al. 1999], the findings of this study support data previously reported from India [Premalatha et al. 2000].

The prevalence of both asymptomatic and symptomatic PAD in this study is similar to previous reports in various populations [Premalatha et al. 2000, Criqui et al. 2005; UK Prospective Diabetes Study Group 1994; Mohan et al. 1995; Chaturvedi et al.2007; Collins

et al. 2005; McDermott et al.2005; Ramos et al. 2009]. Importantly it found no difference in PAD prevalence, or indeed ABPI, between South Asians and Blacks overall. However, South Asian women were found to have statistically significantly lower ABPI than Black women and a greater prevalence of PAD. Of the traditional cardiovascular risk factors investigated in this study, no differences were reported in prevalence of diabetes and hypertension and smoking was significantly lower amongst South Asian women than their black counterparts. In fact, dyslipidaemia was the only less favourable characteristic in the former ethnic group with lower HDL-C and higher triglycerides. The apparent finding of this study could be due to a more sedentary lifestyle which exists amongst South Asian women when compared to Blacks, although this was not measured as part of the study. It might also be due to the higher level of illiteracy in the former group and therefore reduced access to healthcare and primary prevention strategies. It might also be due to genetic differences in predisposition to PAD. However, due to the low numbers of participants and the cross-sectional nature of this study it is impossible to make firm conclusions. Further, better powered studies investigating possible explanations for this study's findings are necessary to find the answer.

This prevalence of PAD in the South Asian subjects differs from the low rate of PAD reported in the only Indian epidemiological study [Premalatha et al. 2000]. However the average age of participants in this study was considerably older and reflects the fact that PAD is predominantly a disease of the elderly. In both the South Asian and Black groups, subjects with PAD were statistically significantly older than those with normal ABPI on univariate analyses, with a greater age difference being found amongst the Black subjects. Indeed age was negatively correlated with ABPI (r= -0.17; p=0.001 and r= -0.157; p=0.021

in Blacks and South Asians respectively). On multivariate analyses age was not an independent predictor of PAD but was a statistically significant predictor of ABPI whereas in Blacks PAD was the most statistically significant independent predictor of PAD but not of ABPI. Clearly age is important in the development of PAD in both ethnic groups; however with increasing age comes the prevalence of more comorbidity which might make independent associations more difficult to find.

Factors which may play a role in explaining apparent differences in PAD prevalence are patient education, access to healthcare and communication issues. It has been thought that the higher prevalence of PAD in African Americans over Whites is due to level of education, income and access to healthcare, and once these are accounted for, African Americans are no more likely to have PAD than Whites [Collins et al.2005]. In the UK, where healthcare is free at the point of access, Black ethnicity has not been found to be associated with higher prevalent PAD than Whites.

Disparities in health and healthcare clearly exist among minority ethnic groups in the UK [Diabetes in the UK, 2004]. It is estimated that there are over 300,000 people from 4 established communities within the UK (Indian, Pakistani, Bangladeshi, and Chinese) unable to converse adequately with their healthcare professional [Gill et al. 2009]. South Asian women in particular maybe more disadvantaged in communicating their problems to health professionals. This may partly explain the lower prevalence of PAD reported previously [UK PDS 1994; Chaturvedi et al. 2007].

Another explanation for the apparent difference in PAD prevalence in South Asian and Black women could be the possibility of a genetic pre-disposition to cardiovascular disease and indeed to the development of vascular disease in different territories. South Asians living in the UK have a higher incidence of and mortality from CAD than Europeans [Balarajan et al. 1991; Balarajan et al. 1996; Raleigh et al. 1997] and Blacks have a higher incidence of and mortality from CBVD and less CAD [Balarajan et al. 1991; Balarajan et al. 1996; Raleigh et al. 1997]. While previous studies have suggested the prevalence of PAD is lower in South Asians [Premalatha et al. 2000; UKPDS 1994; Chaturvedi et al. 2007; Mohan et al. 1995], there are very little epidemiological data on this subject. Further, greater powered, research investigating any apparent differences in risk factor prevalence and their associations with ABPI and PAD are required before firm conclusions can be made regarding whether ethnic differences exist in the prevalence of PAD.

Limitations

ABPI measurement was used as a surrogate indicator of PAD in this study. A value of<0.9 is considered a reliable method for detecting PAD and it has been shown to be 95% sensitive and specific for detecting angiogram positive disease [Dormandy et al. 2000; Hummel et al. 1978; Matzke et al. 2003]. This study was fully intended to be a small pilot study so that preliminary data could be obtained in order to plan a much larger epidemiological study in the UK. As a consequence, participant numbers were small which may have affected associations between ABPI as a continuous variable, PAD and risk factors. Consent for blood was low, especially in South Asians, but comparable to those found in national surveys [Health Survey for England 1999].

Due to the cross-sectional design of this study causal associations between socio-demographic variables and PAD could not be reported. One must therefore be cautious when interpreting the data. Another limitation of this study is that of responder bias. Whilst all eligible South Asian and Black subjects were invited to take part in the E-ECHOES study, patients could respond via a free-phone telephone number or return a stamped addressed envelope. To minimise response bias especially from non-English speakers, subjects were telephoned and a verbal explanation was provided. Another possible bias may come from the fact that ethnic minority participants with co-morbidities, such as hypertension and diabetes, may be more likely to attend for screening than their healthier counterparts, which may have reflected the high prevalence of these co-morbidities in this study. This may also explain the lack of independent associations between traditional cardiovascular risk factors ABPI and PAD on multivariate analyses.

3.2.7 Conclusion

This study in South Asians and Blacks found equivalent rates of PAD to those previously reported in White populations. However the prevalence of PAD in South Asian women was higher and the ABPI was lower than in Black women. These findings could not be explained by the prevalence of traditional cardiovascular risk factors. Further, greater powered, studies are required to see whether socio-demographic factors or genetic markers play the greater role in the development and progression of PAD in different ethnic groups.

3.3 Ethnic differences in Common Carotid Intima-Media Thickness, and the relationship to Cardiovascular Risk Factors and Peripheral Arterial Disease

3.3.1 Abstract

Objectives: To compare the mean and maximum common carotid intima–media thickness (CCIMT) in Blacks (Black Caribbean and Black African) and South Asians (People originating from India, Pakistan and Bangladesh) in a population survey and make associations with established cardiovascular risk factors and peripheral arterial disease (PAD).

Methods: A subset of 492 (293 South Asians and 199 Blacks) out of 572 participants aged ≥45 years recruited in a sub-study to the Ethnic-Echocardiographic Heart of England Screening (E-ECHOES) epidemiological study had mean and maximum CCIMT measured. A questionnaire, anthropometric measurements and Ankle Brachial Pressure Index (ABPI) and Intermittent Claudication assessments were made.

Results: Black participants had greater mean but not maximum CCIMT when compared to South Asians overall, in men and in women on univariate analysis (All $p<0.05$). Black ethnicity was an independent predictor of mean and maximum CCIMT after adjusting for traditional cardiovascular risk factors on multivariate analysis (Both $p<0.05$). After adjusting for age, ethnicity and traditional cardiovascular risk factors, the presence of PAD remained independently predictive of mean ($p=0.019$) and maximum ($p=0.012$) CCIMT on multivariate analysis.

Conclusions: Black ethnicity is related to greater mean and maximum CCIMT when compared to South Asians after adjusting for traditional cardiovascular risk factors. The

presence of PAD independently predicts mean and maximum CCIMT adjusting for ethnicity, age and cardiovascular risk factors.

3.3.2 Introduction

In the UK, South Asians (people originating from India, Pakistan and Bangladesh) and Blacks (Black African and Black Caribbean) have a higher cardiovascular disease burden when compared to white Europeans [Cappuccio et al. 2003]. South Asians in particular have an excess mortality from coronary artery disease (CAD), stroke and end-stage renal disease [Wild et al. 1997; Balarajan et al. 1991] and Blacks a higher mortality from stroke and end-stage renal disease [Wild et al. 1997; Balarajan et al. 1991] than white Europeans. Often, cardiovascular and cerebrovascular events occur in individuals without known pre-existing cardiovascular disease [Fowkes et al. 2008]. It is therefore important to accurately identify those subjects at highest risk in an apparently healthy population, who might benefit from targeted preventative measures [Price et al. 2007].

Non-invasive markers that allow identification of sub-clinical atherosclerosis, include ABPI and carotid artery intima-medial thickness (IMT). An ABPI value <0.9 has long been used as a surrogate marker for peripheral arterial disease (PAD) with 95% sensitive and specificity for detecting angiogram positive disease [Dormandy et al. 2000; Hummel et al. 1978; Matzke et al. 2003]. The ABPI, however, is also an indicator of generalised atherosclerosis being associated with higher rates of CAD and cerebrovascular disease (CBVD) [Newman et al. 1993a], as well as cardiovascular risk factors [Newman et al. 1993a]. Common Carotid artery IMT (CCIMT) is an index of pre-clinical atherosclerosis [D'Agostino et al. 1996]. CCIMT has been associated with conventional cardiovascular risk factors, such as smoking, diabetes mellitus and LDL cholesterol [Markus et al. 2001; Mohan et al. 2000]. An increase in CCIMT is associated with greater cardiovascular endpoints

including stroke, CAD and with both asymptomatic and symptomatic PAD [D'Agostino et al. 1996; Pasternak et al. 2004; Allan et al. 1997; McDermott et al.2005]. Both common and internal CCIMT have been shown to be much higher in patients with PAD, at least in predominantly white European populations [McDermott et al.2005].

Studies have previously shown some ethnic and racial variations in CCIMT [D'Agostino et al. 1996; Markus et al. 2001; McDermott et al. 2005; Zheng et al. 1997; Kuller et al. 1998]. For example, Blacks have significantly greater CCIMT than Whites, after adjustments for cardiovascular risk factors [D'Agostino et al. 1996; Kuller et al. 1998] but no difference or a lower internal carotid artery IMT was found in these studies. A study investigating PAD found no difference in carotid artery IMT between Indian Asians and Europeans [Chaturvedi et al. 2007] in contrast to a previous study [Anand et al. 2000]. Given that South Asians and Blacks comprise 75% of minority ethnic groups in the UK [National Statistics Online]. In order to meet the healthcare needs of the diverse population which exists in the UK, it is important to know if any differences in disease epidemiology exist between different ethnic groups.

The aim of this study was to determine whether any differences in CCIMT exist between the two largest UK minority ethnic groups (South Asians and Blacks) and to see whether its relationships between cardiovascular risk factors and PAD differ. There is currently no agreement as to whether mean CCIMT (an average IMT measured over a 1cm segment of artery) or maximum CCIMT (the highest focal IMT measurement in a 1cm segment) confers the highest cardiovascular risk and so both measurements were taken for analysis.

3.3.3 Hypotheses to be tested

Ethnic differences will exist in mean and maximum CCIMT measurements between South Asians and Blacks.

Ethnic differences will exist between mean and maximum CCIMT and their associations with traditional cardiovascular risk factors and PAD in South Asians and Blacks.

3.3.4 Patients and Methods

Study design and recruitment

Details of the study design and recruitment into the E-ECHOES study and into this sub-study are described in detail in Chapter 2.4.1 2 on page 82.

Clinical Assessment

Demographic and medical history was collected using a standard questionnaire and recording form. Data were derived from both the patient and their medical records. All clinical assessments were performed at the same visit. An in-depth description of the clinical assessment that was undertaken is described in Chapter 2.4.1.3 on page 86.

Common Carotid Artery Intima Medial Thickness (CCIMT) Assessment

CCIMT assessment is described in Chapter 2.4.1.4 on page 88.

PAD Assessment

A description of how PAD was assessed is found in section 2.4.1.6 on page 92.

Blood Sampling

If consent was granted, blood was drawn from the antecubital fossa using the Vacutainer system. Blood was analysed for full blood count, urea and electrolytes, liver function tests, random glucose and random total cholesterol and triglycerides. Consent was obtained for blood sampling in 237 out of 492 (48.2%) of the study cohort (117 out of 292 (39.9%) South Asians and 117 out of 199 (58.8%) Blacks).

Statistical Analysis

Statistical analysis was carried out using Minitab version 15 (State Coll, PA, USA). Data were summarised using mean, median, standard deviation and inter-quartile range for continuous parameters. Student's t-test or the Mann-Whitney U test was used for differences in continuous variables and Chi-squared test for categorical data. Paired t-test was used to compare the values in either side of the neck. Spearman rank correlation coefficient was calculated to test the association between the CCIMT and a number of risk factors. Based on previous reports on the dangers of multiple significance testing in cross-sectional studies, a Bonferroni correction was not performed and a p-value <0.05 was deemed significant. In order to test the hypothesis that ethnic differences will exist in associations between traditional cardiovascular risk factors and mean and maximum CCIMT in South Asians and Blacks, these were entered into multivariate analyses.

3.3.5 Results

Between March 2008 and February 2009, 492 subjects (293 South Asians and 199 Blacks) were recruited into this sub-study to the E-ECHOES study. The mean age of South Asians was 61(12) years and 60.4% were males compared to 62(12) years and 46.7% males in Blacks (p=0.237 and p=0.003 respectively). Of 492 subjects, 7 patients only had CCIMT measurements taken from 1 side, which was used in the analysis - for the remainder - the highest side was used in the subsequent analysis. Due to the lack of consensus as to whether mean or maximum CCIMT is the more important indicator of vascular disease, I investigated possible associations between both of these and traditional cardiovascular risk factors, vascular disease states (PAD, CAD and IC) and treatment for vascular disease (antiplatelet and cholesterol lowering medication).

Table 3.15 shows clinical, demographic and mean and maximum CCIMT characteristics of the two groups. The South Asian group included more men, more 1st generation migrants, greater levels of illiteracy, higher serum triglycerides and lower HDL-cholesterol compared to the Blacks. Black subjects had a higher proportion of current and ever smokers and higher body mass index (BMI) than South Asians.

Table 3.15: Clinical, demographic and CCIMT characteristics by Ethnic Group

Variable	South Asian* n=293 (SD)	Blacks n=199 (SD)	p-value
Age (years)	61 (12)	62 (12)	0.237
Male (%)	60.4	46.7	0.003
1st generation migrants (%)	98.6	84.9	<0.0001
Illiteracy (%)	27.9	0	<0.0001
BMI	27.8 (4.64)	29.5 (5.75)	0.001
Waist circumference (cm)	97.8 (12.6)	97.6 (12.7)	0.839
SBP (mmHg)	143 (20)	144.5 (18)	0.377
DBP (mmHg)	82 (11)	83 (10)	0.495
PP (mmHg)	61 (16)	62 (16)	0.543
PAD (%)	14.7	10.6	0.182
CAD (%)	14	7	0.016
CBVD (%)	3.8	4.5	0.672
Diabetes (%)	34.8	28.1	0.12
Hypertension (%)	71.3	73.9	0.537
Antiplatelet medication usage (%)	36.5	31.7	0.266
Cholesterol lowering medication (%)	49.1	41.7	0.104
Never Smoker (%)	71.3	51.8	<0.0001
Ex-smoker (%)	16.7	26.1	0.011
Current Smoker (%)	12	22.1	0.003
Triglycerides (mmol/l)	1.85 [1.3-2.7] ¥	1.34 [0.8-2.2] к	0.0002
LDL-C (mmol/l)	2.31 [1.8-2.6] ¥	2.53 [1.8-3.1] к	0.339
HDL-C (mmol/l)	1.11 (0.37) ¥	1.37 (0.45) к	<0.0001
Total Cholesterol (mmol/l)	4.5 (1.1) ¥	4.6 (1.1) к	0.413
HbA1c (%)	6.7 (1.2) ч	6.4 (1.5) ж	0.116
Mean CCIMT (mm)	0.61 (0.13)	0.64 (0.14)	0.022
Maximum CCIMT (mm)	0.73 (0.16)	0.75 (0.16)	0.113

SD: standard deviation; SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin,; PAD: peripheral arterial disease; CAD: coronary artery disease; CBVD: cerebrovascular disease; CCIMT: common carotid intima medial thickness; ¥: 117; к: n=115; ч : n=120; ж : n=117

*The ethnic breakdown of the South Asian cohort was 40.4% Pakistani, 38.9% Indian, 16.9% Bangladeshi and 2.8% East African Asian, which is representative of the distribution of these ethnic groups within Birmingham [Population Estimates by Ethnic Group 2007].

South Asians:

Analysis according to sex is illustrated in table 3.16. South Asian women had a statistically significantly lower prevalence of hypertension (p=0.01), diabetes (p=0.036) and were less likely to have ever smoked (p<0.0001) or be current smokers (p<0.0001) than men. They were also less likely to be taking antiplatelets (p=0.001), with a non-statistically significant trend towards lower usage of cholesterol lowering medication (p=0.056). South Asian men had a statistically significantly lower mean (p=0.001) and maximum (p=0.005) CCIMT than South Asian men.

In an attempt to establish which of mean and maximum CCIMT better reflected a greater cardiovascular disease burden, overt vascular disease and possible effects of treatment I investigated these associations with univariate and multivariate analyses. Associations between categorical and continuous variables on univariate analysis are shown in table 3.17 and 3.18 respectively. Of the traditional cardiovascular risk factors, the presence of hypertension was significantly associated with higher mean and maximum CCIMT (p<0.0001 for both) and both SBP and PP correlated strongly with both of these (all p<0.0001) on univariate analyses. Prevalent diabetes and HbA1c were also associated with both mean (p=0.044 and p=0.022 respectively) and maximum (p=0.047 and 0.042 respectively) CCIMT on univariate analyses as was having ever smoked (p=0.002 (mean) and p=0.018(maximum)).

Of the continuous variables studied, SBP and PP (discussed above), age (p<0.0001 for both), total cholesterol (p=0.006 (mean) and p=0.012 (maximum)) and LDL-C (p=0.007 (mean) and p=0.003 (maximum)) were significantly associated with both mean and maximum

CCIMT on univariate analyses. Correlations between continuous variables and mean and maximum CCIMT are displayed graphically in figures 3.5-3.10. In this study SBP was strongly correlated with PP ($r=0.83$; $p<0.0001$).

The presence of co-existent PAD was statistically significantly associated with higher mean ($p=0.035$) and maximum ($p=0.003$) CCIMT on univariate analyses and the presence of co-existent CAD was associated with greater mean CCIMT ($p=0.043$) with a non-significant trend for a higher maximum CCIMT ($p=0.079$).

Patients are usually started on antiplatelet and cholesterol lowering medication when they either have overt cardiovascular disease or are at high cardiovascular risk. One would expect that subjects taking these medications would therefore have greater mean and maximum CCIMT, a marker of pre-clinical atherosclerosis, which was found in this study (All $p<0.05$).

Table 3.16: Characteristics of the South Asian group by sex

Variable	South Asian Male (SD) [IQR] (n= 177)	South Asian Female (SD) [IQR] (n=116)	p-value
Age (years)	61 (11)	61 (12)	0.56
BMI	27.2 (4.3)	28.7 (5.00)	0.006
SBP (mmHg)	144 (19)	141 (21)	0.133
DBP (mmHg)	84 (11)	80 (11)	0.002
PP (mmHg)	60 (15)	61 (17)	0.837
Hypertension (%)	76.8	62.9	0.01
Never Smoker (%)	54.8	96.6	<0.0001
Ex-Smoker >1 year (%)	26.6	1.7	<0.0001
Current Smoker	18.6	1.7	<0.0001
Diabetes (%)	39.5	27.6	0.036
HbA1c	6.9 (1.2) [к]	6.3 (1.1) [ч]	0.023
Total Cholesterol	4.5 (1.1) [¥]	4.6 (1.0) [ч]	0.399
LDL-C	2.24 [1.7-2.8] [¥]	2.44 [2-3] [ч]	0.145
HDL-C	1.04 (0.33) [¥]	1.24 (0.39) [ч]	0.006
Triglycerides	1.95 [1.3-2.8] [¥]	1.74 [1.2-2.6] [ч]	0.313
Cholesterol Lowering medication (%)	53.7	42.2	0.056
Antiplatelet medication (%)	44.1	25	0.001
CAD (%)	16.9	10.3	0.115
CBVD (%)	4.5	2.6	0.394
PAD (%)	12	19	0.093
mean IMT	0.63 (0.14)	0.58 (0.12)	0.001
max IMT	0.75 (0.16)	0.7 (0.15)	0.005

SD: standard deviation; IQR; inter-quartile range; SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin; CAD: coronary artery disease; CBVD: cerebrovascular disease; CCIMT: common carotid intima medial thickness; Max: maximum. [¥]: n=76; [к]: n=79; [ч]: n=41;

Table 3.17: Categorical variables associated with mean and maximum CCIMT in South Asians on univariate analysis

Variable			Maximum CCIMT(mm) (SD)	p- value	Mean CCIMT (mm) (SD)	p-value
Cardiovascular risk factors		N				
Diabetes	Yes	102	0.76 (0.17)		0.63 (0.14)	
	No	191	0.72 (0.15)	0.047	0.6 (0.13)	0.044
Ever smoker	Yes	84	0.77 (0.16)		0.65 (0.14)	
	No	209	0.72 (0.16)	0.018	0.59 (0.13)	0.002
Hypertension	Yes	209	0.76 (0.15)		0.63 (0.13)	
	No	84	0.66 (0.16)	<0.0001	0.56 (0.12)	<0.0001
Cardiovascular disease states						
CAD	Yes	42	0.77 (0.16)		0.65 (0.13)	
	No	251	0.72 (0.16)	0.079	0.6 (0.13)	0.043
PAD	Yes	43	0.79 (0.19)		0.66 (0.16)	
	No	250	0.72 (0.15)	0.03	0.6 (0.13)	0.035
Vascular disease treatment						
Use of cholesterol lowering drugs	Yes	144	0.75 (0.15)		0.63 (0.13)	
	No	149	0.71 (0.17)	0.023	0.59 (0.13)	0.008
Use of antiplatelet medication	Yes	107	0.76 (0.16)		0.64 (0.14)	
	No	186	0.71 (0.14)	0.014	0.59 (0.13)	0.012

Values are mean (SD); SD: standard deviation; PAD: peripheral arterial disease; CAD: coronary arterial disease; IC: intermittent claudication

Table 3.18 illustrates univariate correlations between continuous variables and mean and maximum CCIMT in South Asians. Age, SBP, PP, Total cholesterol, LDL-C, HbA1c all correlated with mean and maximum CCIMT.

Table 3.18: Correlations between continuous variables and CCIMT in South Asians on univariate analysis

Variable	r-value	mean IMT p-value	r-value	max IMT p-value
Age	0.39	<0.0001	0.32	<0.0001
SBP	0.22	<0.0001	0.21	<0.0001
PP	0.26	<0.0001	0.23	<0.0001
Total Cholesterol	-0.25	0.006	-0.23	0.012
LDL-C	-0.25	0.007	-0.28	0.003
HDL-C	-0.13	0.167	-0.14	0.127
Triglycerides	-0.02	0.814	-0.01	0.924
HbA1c	0.19	0.042	0.21	0.022
ABPI	-0.10	0.097	-0.11	0.073

SBP: systolic blood pressure; PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin; CCIMT: common carotid intima medial thickness; Max: maximum; ABPI: ankle brachial pressure index.

Table 3.19 illustrates variables entered into a linear regression model as possible independent predictors of mean and maximum CCIMT in South Asians. Adjusting for traditional cardiovascular risk factors, the following variables were entered into the multivariate analysis: Sex, age, SBP, diabetes, smoking. Due to the low level of blood sampling, cholesterol (Total, LDL or HDL) and HbA1c were not adjusted for or entered into the model. Of the variables entered into multivariate analysis, age and SBP were independent predictors of both mean and maximum CCIMT and smoking was an independent predictor of mean CCIMT.

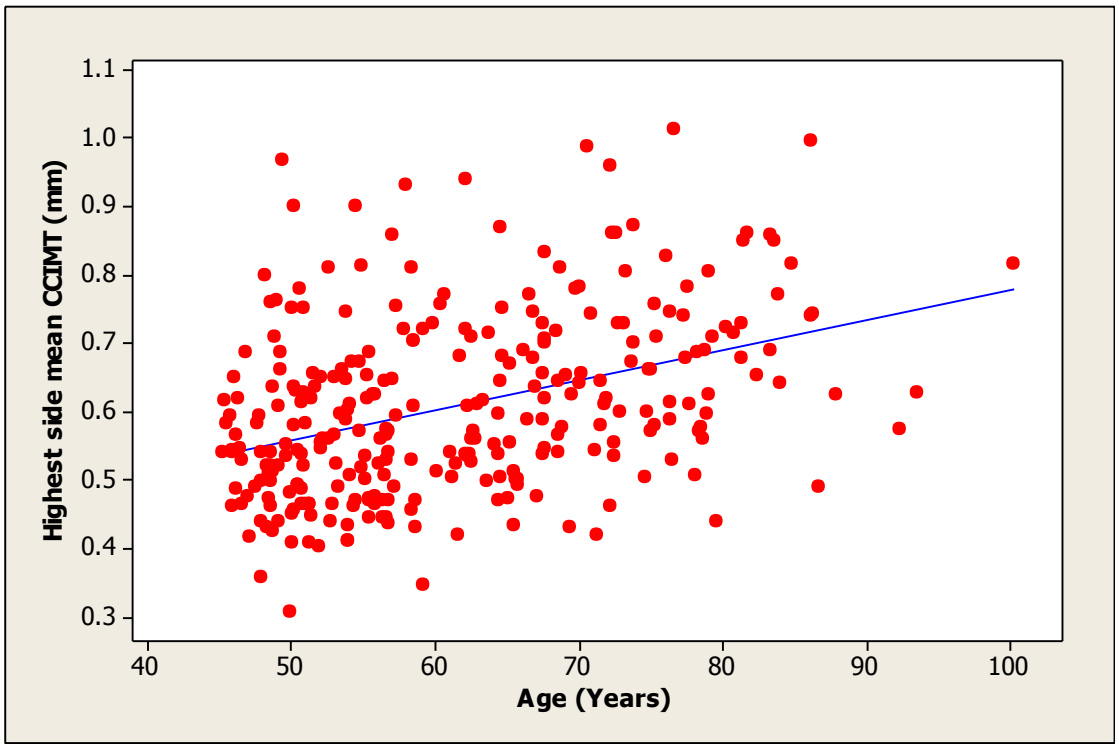
Table 3.19: Variables entered into multivariate analysis as possible independent predictors of mean and maximum CCIMT in South Asians

Variable	Mean CCIMT p-value	Max CCIMT p-value
Age	<0.0001	<0.0001
Smoking	0.046	0.288
SBP	0.048	0.049
Sex	0.066	0.07
Diabetes	0.336	0.274

SBP: systolic blood pressure

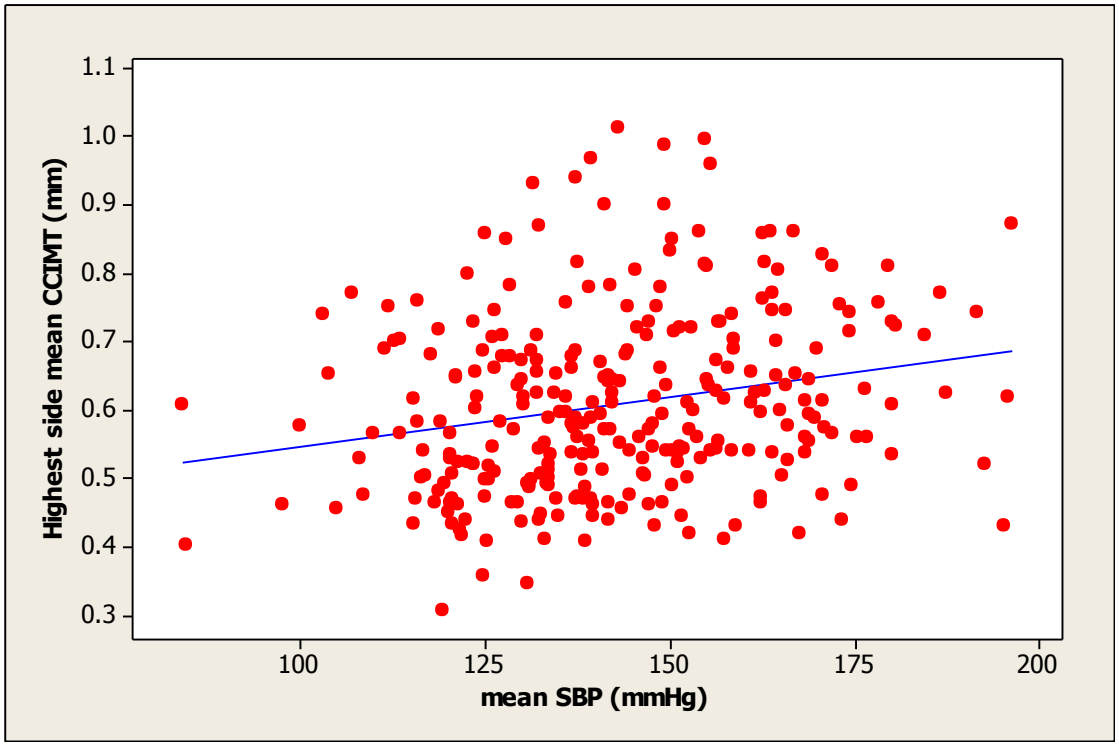
The following figures illustrate significant univariate correlations between continuous variables and mean CCIMT in South Asians.

Figure 3.5: Correlation between mean CCIMT vs. age in South Asians



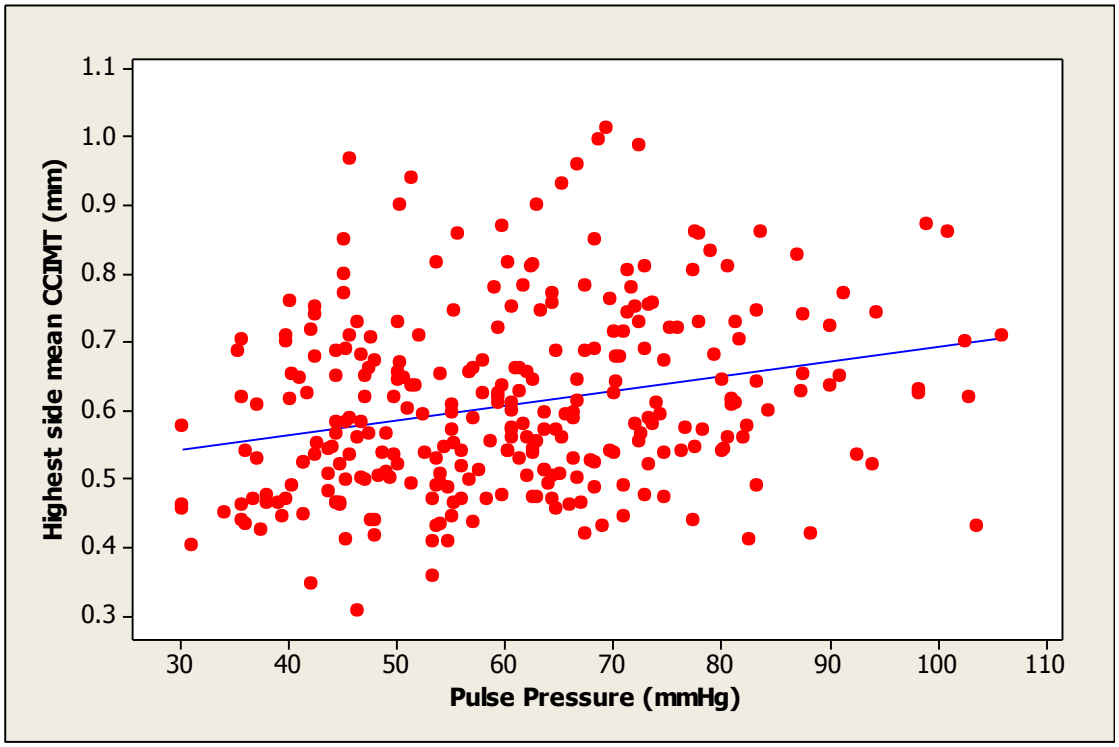
r = 0.39; p<0.0001

Figure 3.6: Correlation between mean CCIMT vs. mean Systolic Blood Pressure in South Asians



r = 0.22; p<0.0001

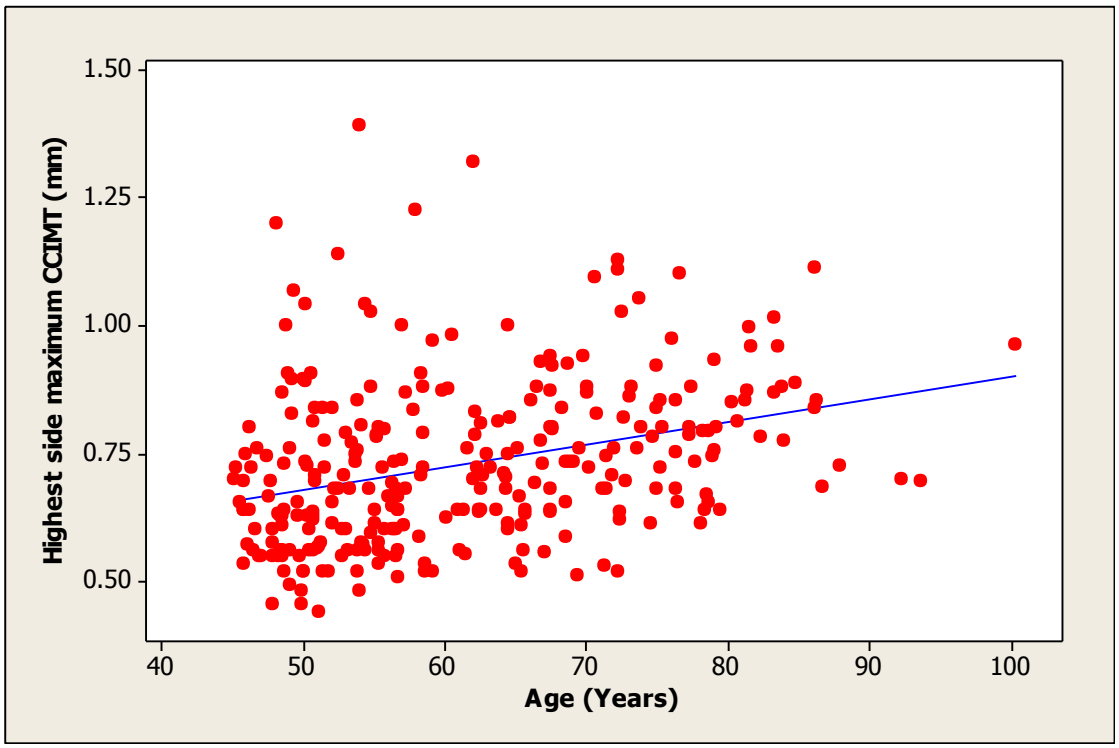
Figure 3.7: Correlation between mean CCIMT vs. Pulse Pressure in South Asians



r = 0.26; p<0.0001

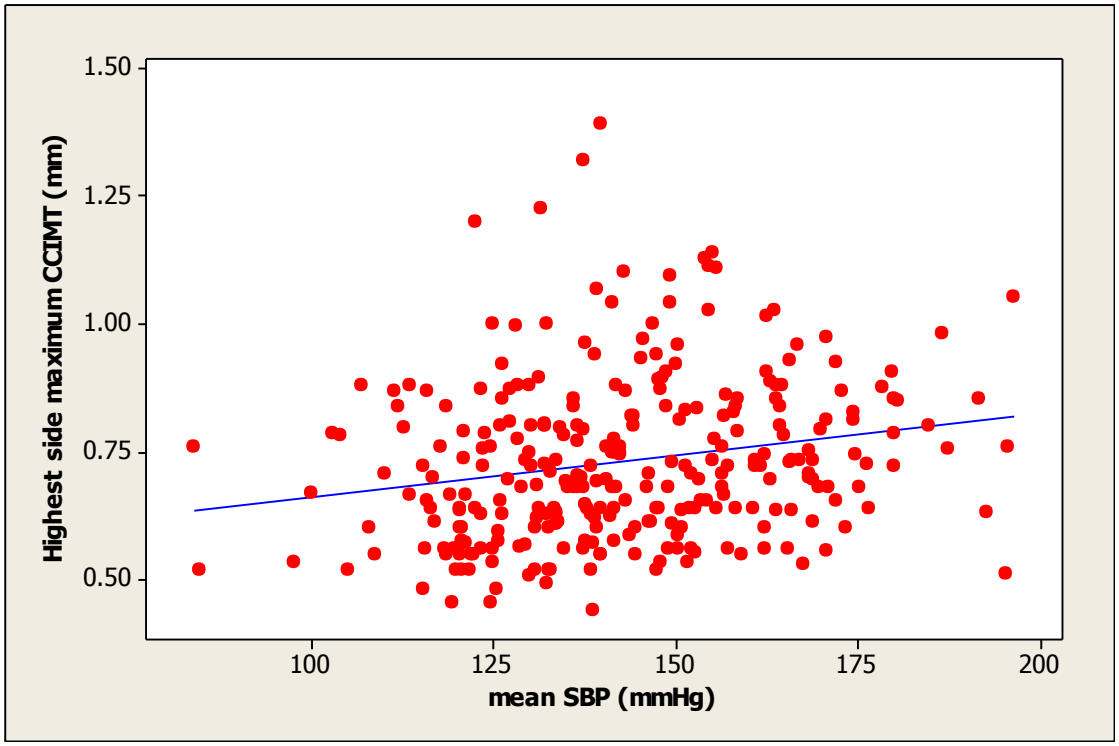
The following figures illustrate significant correlations between continuous variables and maximum CCIMT in South Asians.

Figure 3.8: Correlation between maximum CCIMT vs. Age in South Asians



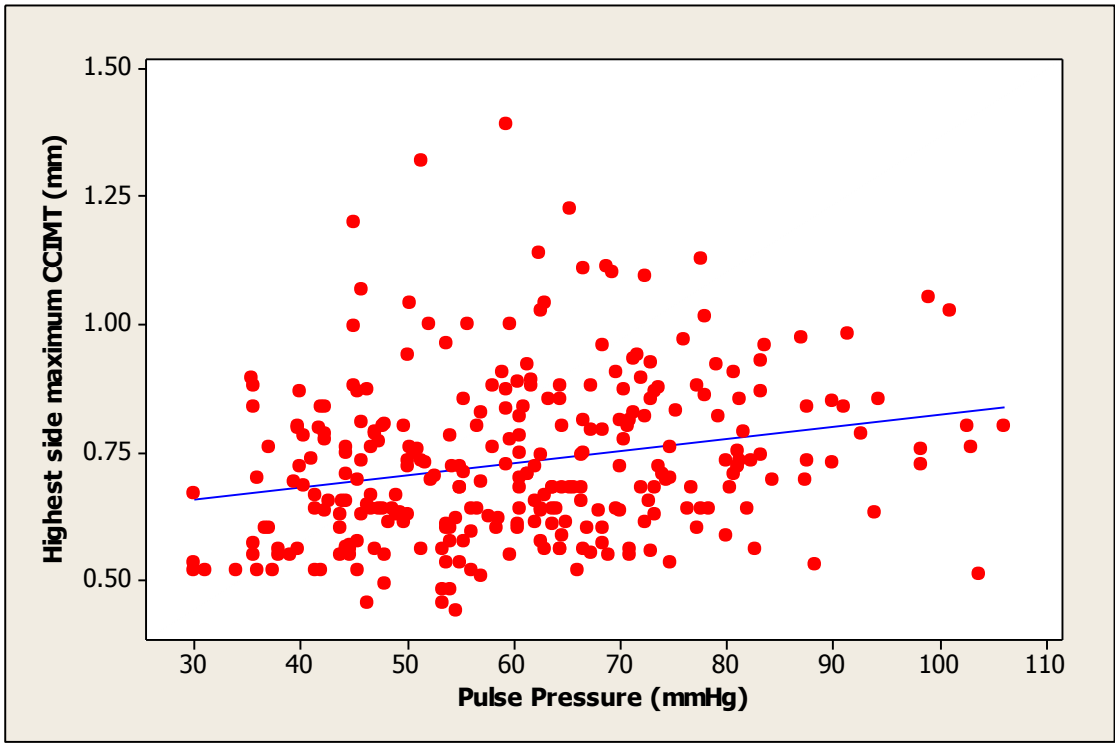
r = 0.32; p<0.0001

Figure 3.9: Correlation between maximum CCIMT vs. mean Systolic Blood Pressure in South Asians



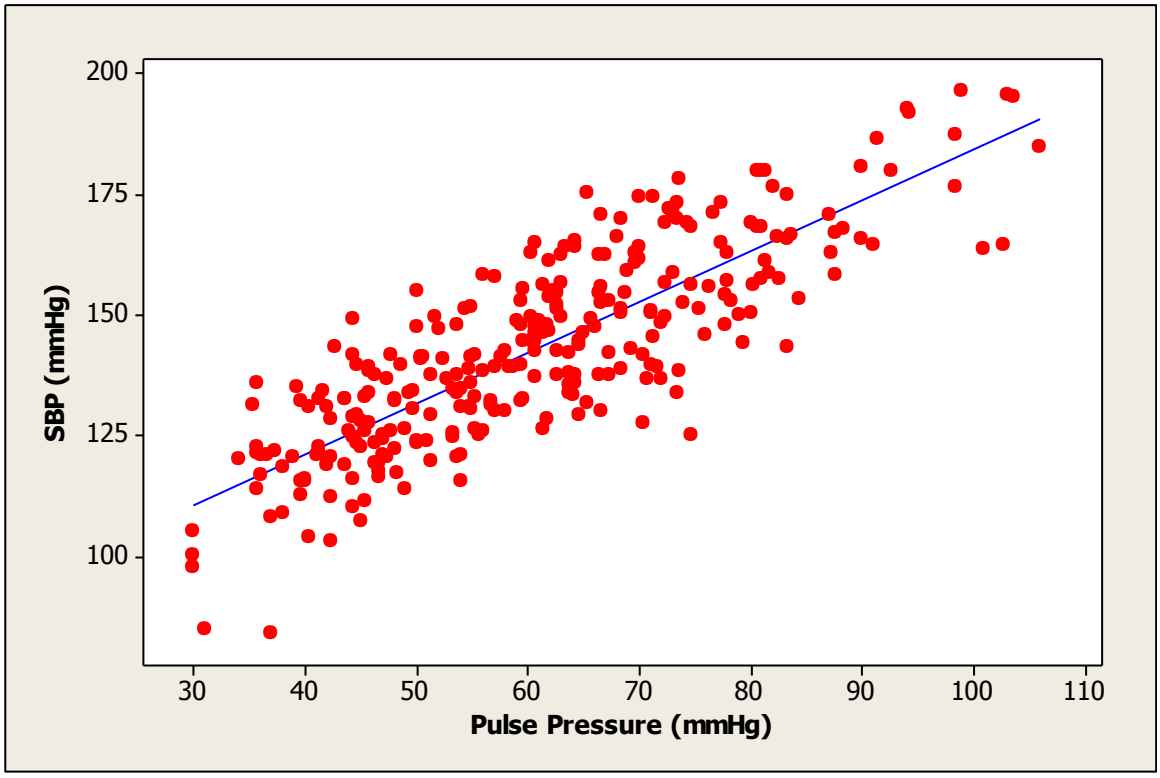
r = 0.21; p<0.0001

Figure 3.10: Correlation between maximum CCIMT vs. Pulse Pressure in South Asians



r = 0.23; p<0.0001

Figure 3.11: Correlation between SBP and PP in South Asians



r = 0.83; p<0.0001

Blacks:

Analysis according to sex is present in table 3.20. Black women had statistically significantly higher BMI than Black men ($p<0.0001$) and were less likely to have ever smoked ($p<0.0001$). No differences were found in prevalence of hypertension or diabetes. Black women had statistically significantly lower mean ($p=0.008$) and maximum ($p=0.01$) CCIMT than Black men.

Univariate associations between categorical and continuous variables and mean and maximum CCIMT are illustrated in tables 3.21 and 3.22 respectively. Of the traditional cardiovascular risk factors, diabetes was statistically significantly associated with mean ($p=0.004$) and maximum ($p=0.011$) CCIMT and HbA1c was positively correlated with them both ($p=0.003$ and $p=0.004$ respectively). Whilst hypertension was not associated with significantly higher mean and maximum CCIMT per se, SBP ($p=0.004$ (mean); $p=0.003$ (maximum)) and PP ($p<0.0001$ (mean); $p<0.0001$ (maximum)) were both statistically significantly associated with them on univariate analysis. Smoking was not statistically significantly associated with higher CCIMT in Blacks.

Amongst the vascular disease states, PAD was significantly associated with higher mean ($p=0.022$) and maximum ($p=0.033$) CCIMT. As with the South Asians this study found that people taking cholesterol lowering medication ($p=0.002$ (mean); $p=0.003$(maximum)) and antiplatelets ($p=0.001$ (mean); $p=0.003$ (maximum)) had higher CCIMT on univariate analysis.

Table 3.20: Characteristics of Black subjects by sex

Variable	Black Male (SD) [IQR] (n=93)	Black Female (SD) [IQR] (n=106)	p-value
Age (years)	63 (11)	61 (11)	0.214
BMI	27.8 (4.5)	31 (6.3)	<0.0001
SBP (mmHg)	147 (18)	143 (18)	0.092
DBP (mmHg)	83 (10)	83 (10)	0.709
PP (mmHg)	64 (15)	60 (16)	0.087
Hypertension (%)	77.4	70.8	0.286
Never Smoker (%)	31.2	69.8	<0.0001
Ex-smoker (%)	35.5	17.9	0.005
Current Smoker (%)	33.3	12.3	<0.0001
Diabetes (%)	29	26	0.645
HbA1c	6.5 (1.3) [Ə]	6.3 (1.6) [#]	0.351
Total Cholesterol	4.4 (1.1) [ж]	4.8 (1.1) [^]	0.028
LDL-C	2.2 [1.6-2.9] [ж]	2.7 [2-3.1] [^]	0.04
HDL-C	1.24 [0.9-1.5] [ж]	1.45 (0.45) [^]	0.04
Triglycerides	1.3 [0.9-2.3] [ж]	1.43 [0.7-2.0] [^]	0.78
Cholesterol Lowering medication (%)	45.2	38.7	0.355
Antiplatelet medication (%)	36.6	27.4	0.164
CAD (%)	5.4	8.5	0.391
CBVD (%)	4.3	4.7	0.888
PAD (%)	15.1	6.6	0.053
mean IMT (mm)	0.67 (0.14)	0.61 (0.13)	0.008
max IMT (mm)	0.79 (0.17)	0.73 (0.15)	0.01

SD: standard deviation; IQR; inter-quartile range; SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin; CAD: coronary artery disease; CBVD: cerebrovascular disease; CCIMT: common carotid intima medial thickness; Max: maximum. [¥]: n=76; [ĸ]: n=79; [ч]: n=41; [ж]: n=47; [Ə] : n=51; [^] : n=68; [#] : n=66.

Table 3.21: Categorical variables and CCIMT in Blacks

Variable			Maximum CCIMT(mm) (SD)	p-value	Mean CCIMT (mm) (SD)	p-value
Cardiovascular risk factors		N				
Diabetes	Yes	56	0.81 (0.17)		0.68 (0.15)	
	No	143	0.73 (0.16)	0.004	0.62 (0.13)	0.011
Ever smoker	Yes	96	0.76 (0.18)		0.64 (0.15)	
	No	103	0.75 (0.15)	0.743	0.63 (0.13)	0.542
Hypertension	Yes	147	0.76 (0.17)		0.64 (0.14)	
	No	52	0.73 (0.15)	0.186	0.62 (0.12)	0.187
Cardiovascular disease states						
CAD	Yes	14	0.85 (0.26)		0.72 (0.21)	
	No	185	0.75 (0.15)	0.172	0.63 (0.13)	0.154
PAD	Yes	21	0.86 (0.2)		0.72 (0.18)	
	No	178	0.74 (0.15)	0.022	0.62 (0.13)	0.033
Vascular disease treatment						
Cholesterol Lowering Medication	Yes	83	0.8 (0.18)		0.67 (0.15)	
	No	116	0.72 (0.15)	0.002	0.61 (0.13)	0.003
Antiplatelets	Yes	63	0.81 (0.17)		0.68 (0.14)	
	No	136	0.73 (0.16)	0.001	0.62 (0.13)	0.003

Values are mean (SD); SD: standard deviation; PAD: peripheral arterial disease; CAD: coronary arterial disease; IC: intermittent claudication

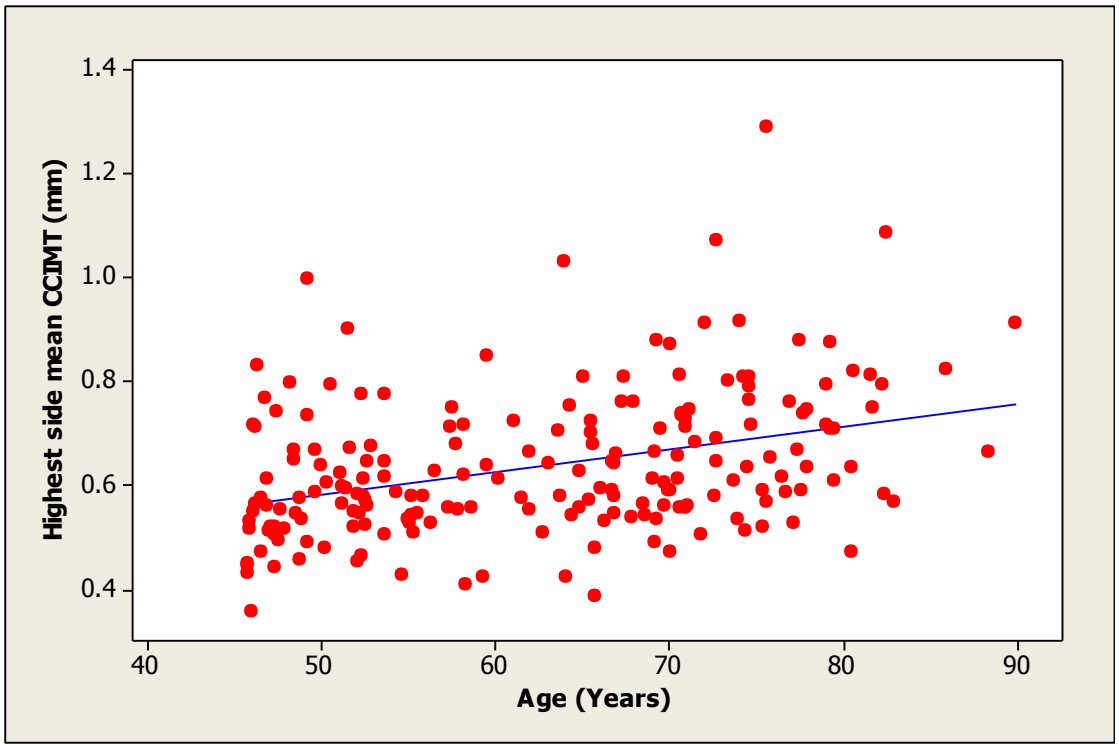
Table 3.22: Correlations between continuous variables and CCIMT in Blacks on univariate analysis

Variables	r-value	mean IMT p-value	r-value	max IMT p-value
Age	0.36	<0.001	0.38	<0.0001
SBP	0.20	0.004	0.21	0.003
PP	0.29	<0.0001	0.30	<0.0001
Total Cholesterol	-0.02	0.863	-0.002	0.981
LDL-C	-0.06	0.556	-0.06	0.484
HDL-C	-0.01	0.955	-0.05	0.609
Triglycerides	0.03	0.775	0.09	0.354
HbA1c	0.27	0.003	0.26	0.004
ABPI	-0.10	0.146	-0.10	0.146

SBP: systolic blood pressure; PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin; CCIMT: common carotid intima medial thickness; Max: maximum; ABPI: ankle brachial pressure index.

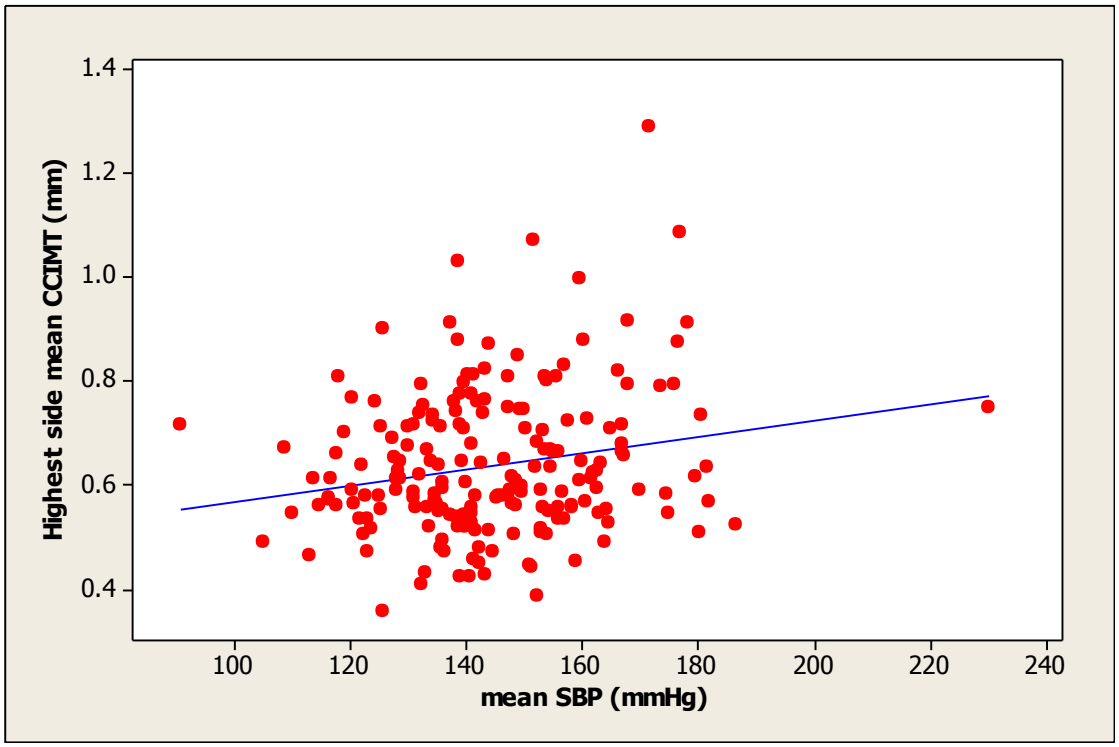
Univariate correlations between variables and mean and maximum CCIMT in Blacks are displayed graphically in figures 3.12-3.17.

Figure 3.12: Correlation between mean CCIMT vs. Age in Blacks



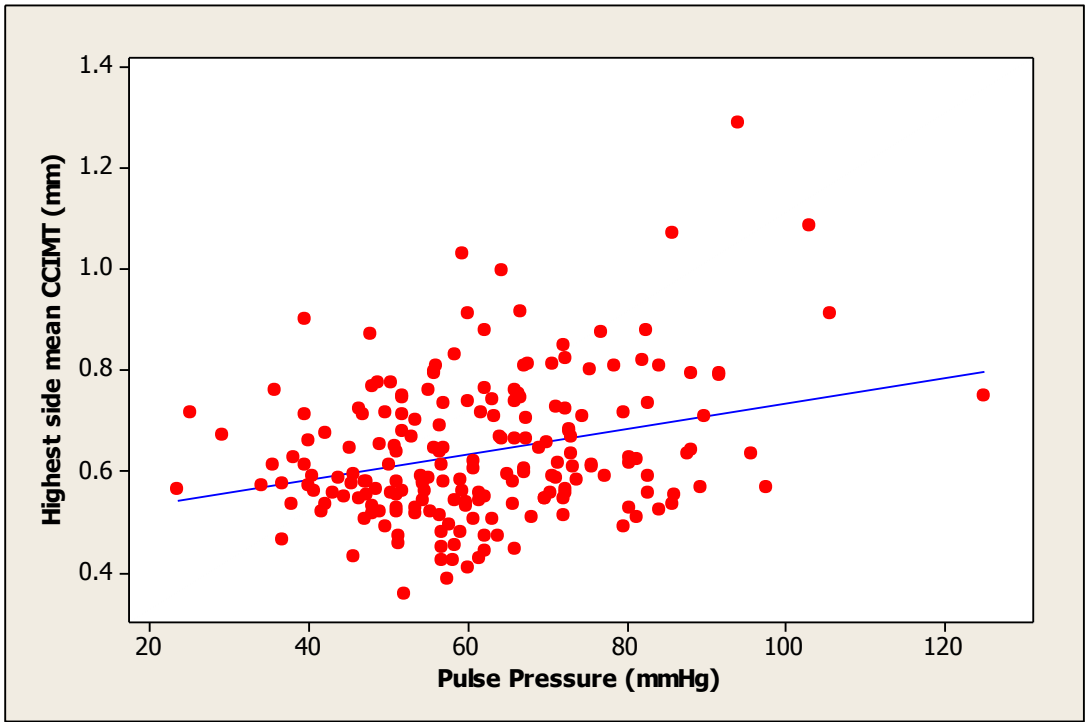
r = 0.36; p<0.0001

Figure 3.13: Correlation between mean CCIMT vs. mean Systolic Blood Pressure in Blacks



r = 0.20; p = 0.004

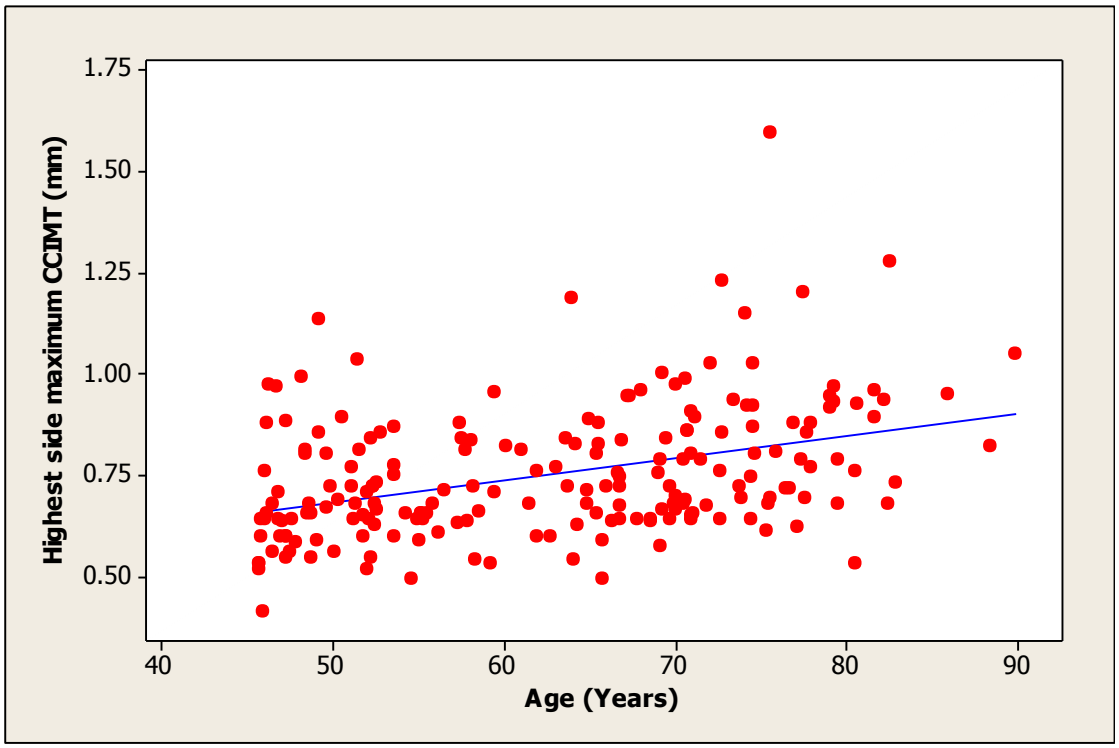
Figure 3.14: Correlation between mean CCIMT vs. Pulse Pressure in Blacks



r = 0.29; p<0.0001

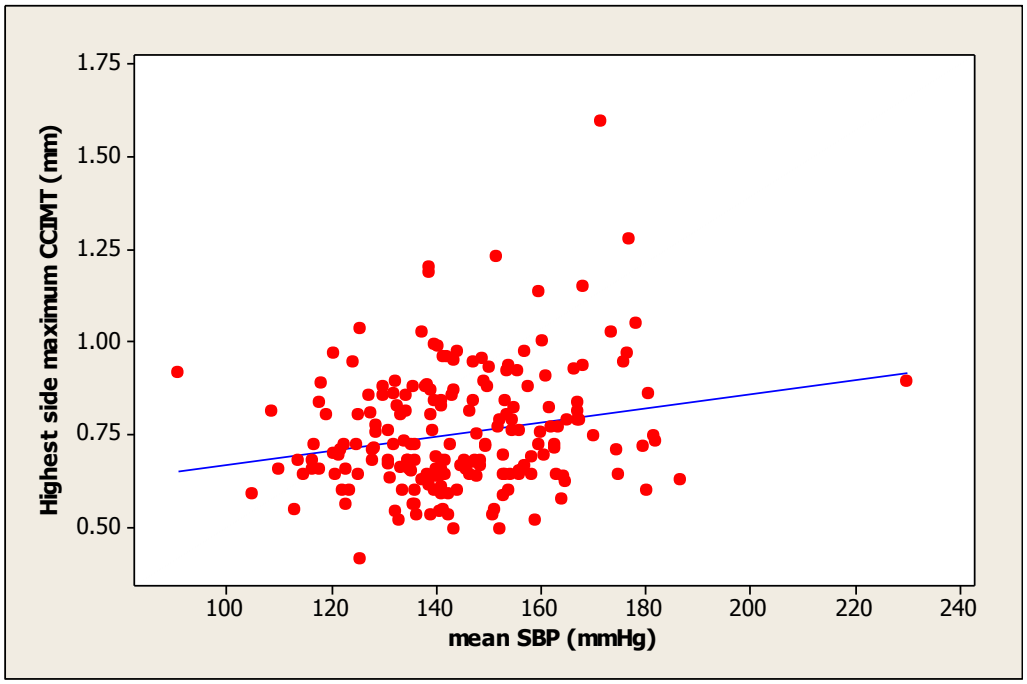
The following figures illustrate univariate correlations between continuous variables and maximum CCIMT in Blacks.

Figure 3.15: Correlation between maximum CCIMT vs. Age in Blacks



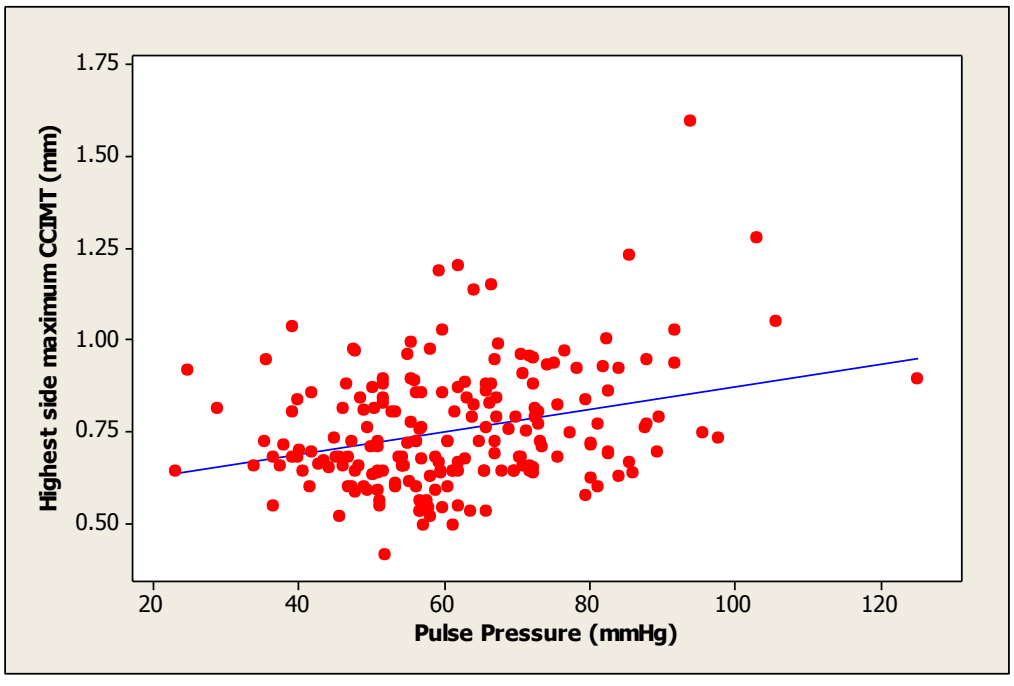
r = 0.38; p<0.0001

Figure 3.16: Correlation between maximum CCIMT vs. mean Systolic Blood Pressure in Blacks



r = 0.21; p = 0.003

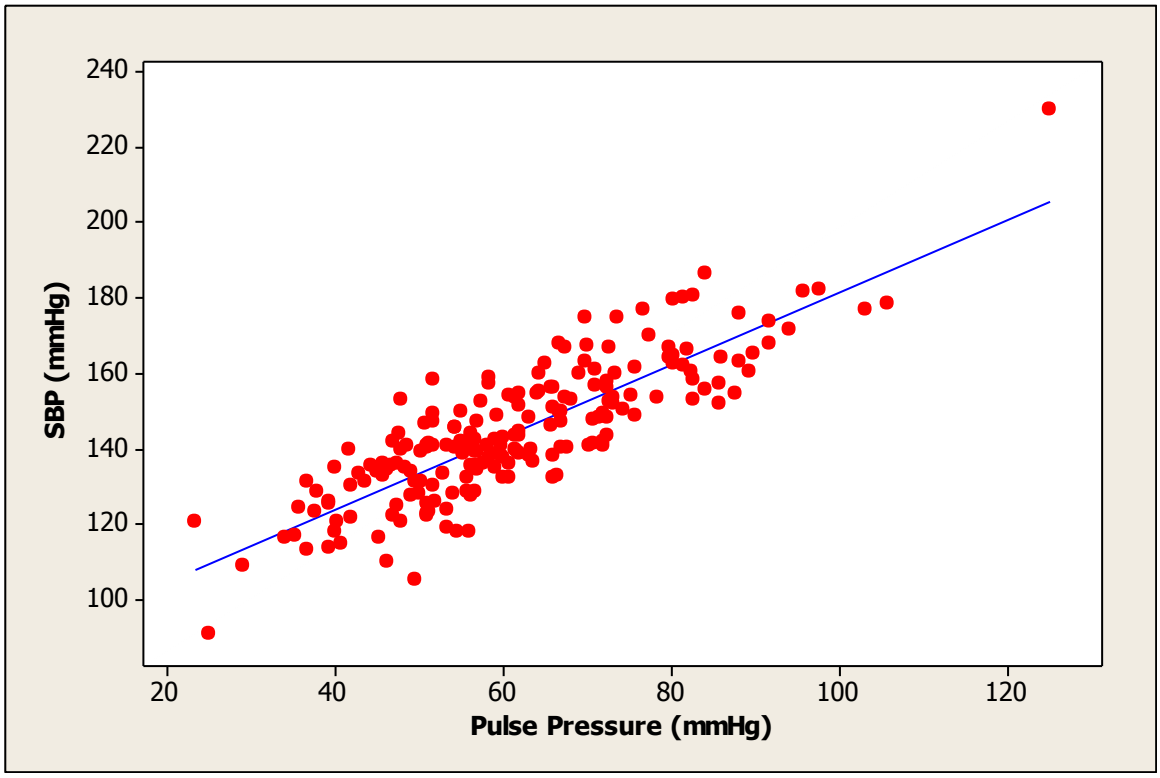
Figure 3.17: Correlation between maximum CCIMT vs. Pulse Pressure in Blacks



r = 0.30; p<0.0001

Figure 3.18 illustrates that SBP correlates strongly with PP in Black subjects.

Figure 3.18: Correlation between SBP and PP in Blacks



r=0.844; p<0.0001

Adjusting for traditional cardiovascular risk factors, the following variables were entered into the multivariate analysis: Sex, age, SBP, diabetes, smoking. Due to the low level of blood sampling, cholesterol (Total, LDL or HDL) and HbA1c were not adjusted for or entered into the model. Results of the multivariate analysis are shown in table 3.23. Only age was a statistically significant predictor of mean and maximum CCIMT.

Table 3.23: Variables entered into multivariate analysis as possible independent predictors of mean and maximum CCIMT in Blacks

Variable	Mean CCIMT p-value	Maximum CCIMT p-value
Age	<0.0001	<0.0001
Sex	0.055	0.060
Diabetes	0.099	0.055
SBP	0.275	0.264
Smoking	0.793	0.900

SBP: systolic blood pressure

Comparing South Asians and Blacks:

On univariate analysis when comparing South Asians and Blacks, mean CCIMT differed significantly when compared overall and by sex (Figure 3.19). Black subjects overall had higher mean CCIMT (0.64 vs. 0.61mm; p=0.022) than South Asians, as did both the male (0.67 vs. 0.63mm; p=0.04) and female (0.61 vs. 0.58mm; p=0.044) subjects. There was no difference in maximum CCIMT between the ethnic groups overall, or between the male or female groups (Figure 3.20).

In an attempt to see if ethnicity is an independent predictor for mean and maximum CCIMT it was entered into a multivariate model, adjusting for traditional cardiovascular risk factors and found ethnicity to be a statistically significant independent predictor of both maximum (p=0.036) and mean (p=0.02) CCIMT.

Because CCIMT is a marker of pre-clinical atherosclerosis this study wanted to investigate if having cardiovascular disease, in the form of PAD was associated with higher mean and maximum CCIMT. Univariate comparisons between subjects of each ethnic group with and without PAD and mean and maximum CCIMT are illustrated in figure 3.21. In order to see if PAD was a significant predictor of mean and maximum CCIMT independent of ethnicity and the presence of cardiovascular risk factors this study entered it into a multivariate model adjusting for traditional risk factors and found that PAD was a statistically significant independent predictor of both mean (p=0.019) and maximum (p=0.012) CCIMT. When adjusting for age, the strongest predictor of CCIMT in both South Asians and Blacks, PAD was still a statistically significant independent predictor of mean (p=0.04) and maximum (p=0.026) CCIMT.

Figure 3.19: Interval Plot illustrating mean CCIMT by sex and ethnicity

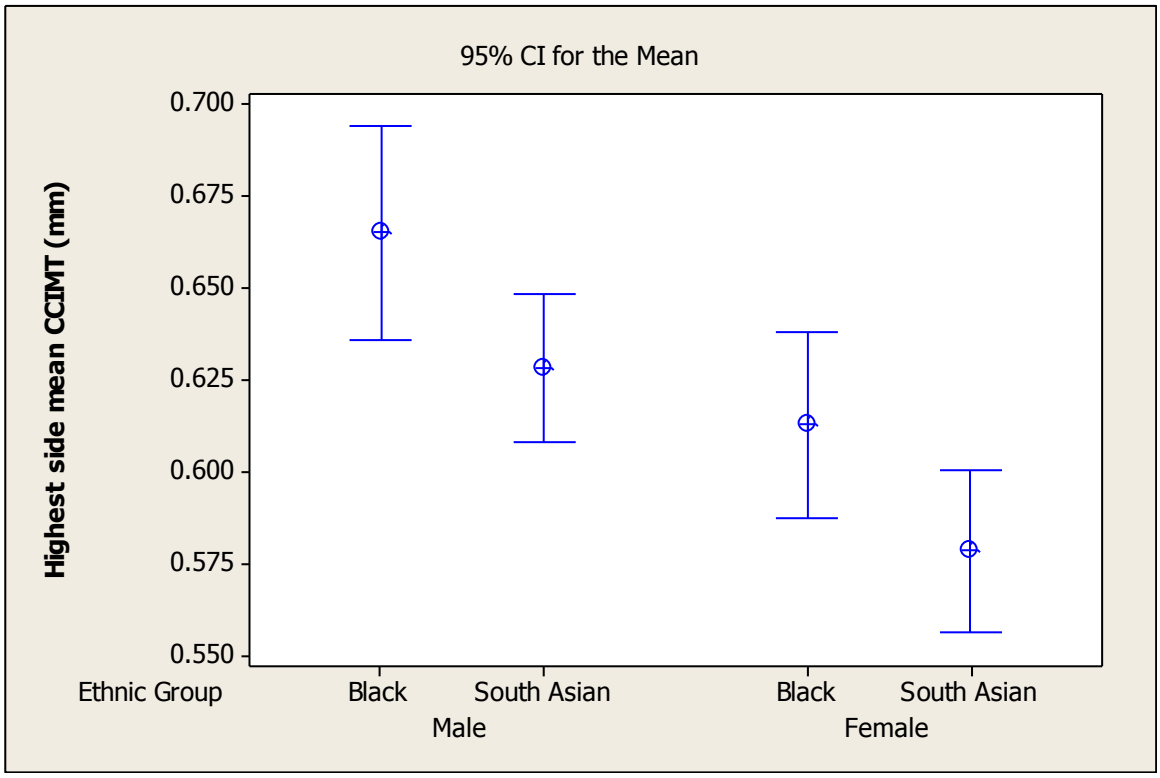


Figure 3.20: Interval Plot illustrating maximum CCIMT by sex and ethnicity

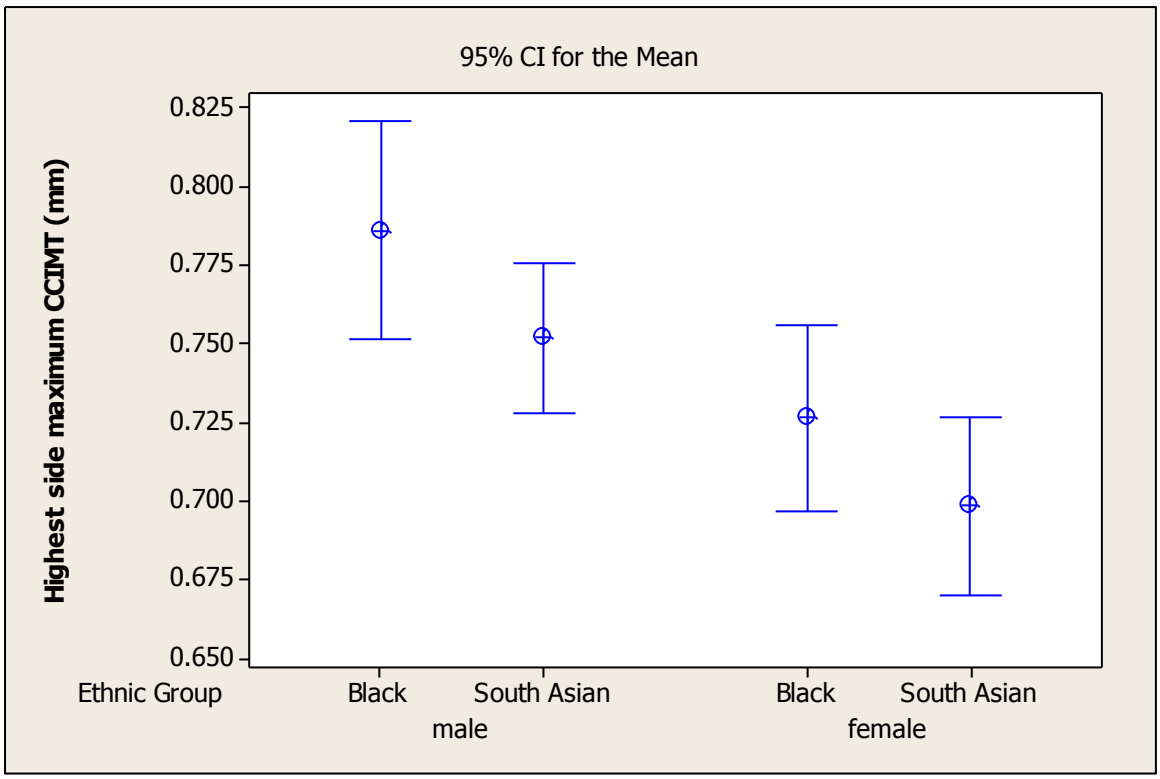
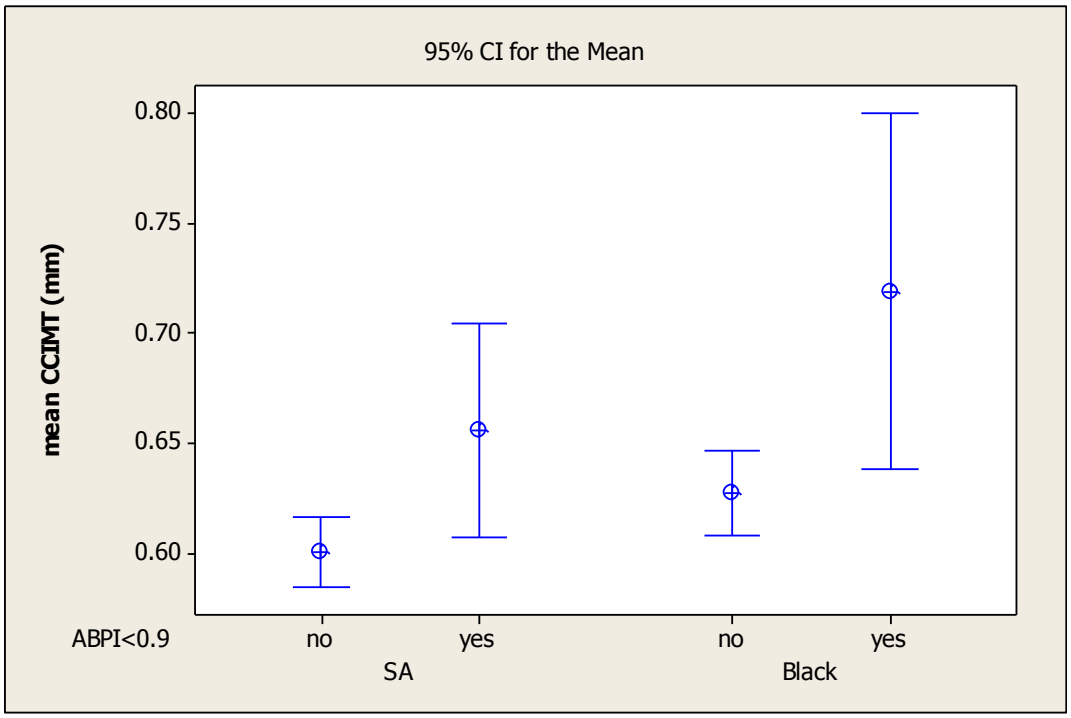
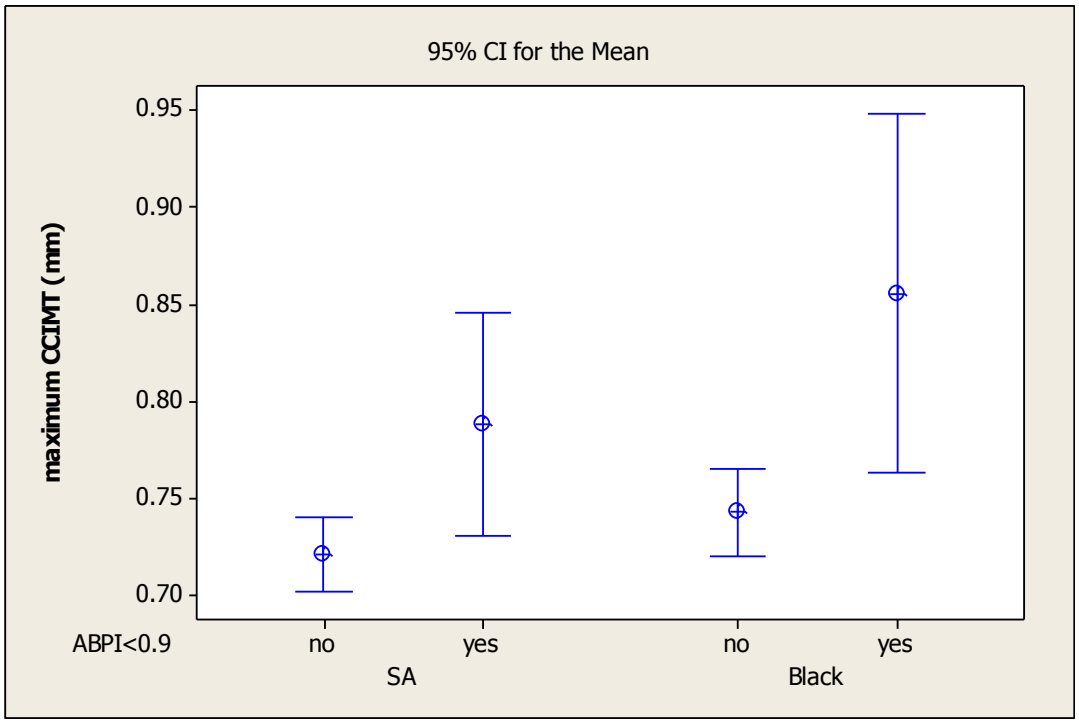


Figure 3.21: Interval Plot illustrating CCIMT (3.22.1: mean; 3.22.2: maximum) in South Asians and Blacks with and without PAD (Based upon ABPI<0.9)

3.21.1



3.21.2



3.3.6 Discussion

This study reports comparisons between CCIMT, cardiovascular risk factors and PAD in predominantly first generation UK South Asian and Black migrants. There is no generalised consensus on whether mean or maximum (focal) thickness is of more importance in determining cardiovascular risk but I suggest that the mean CCIMT over a 1cm segment may better reflect generalised atherosclerosis than CCIMT at a focal point. This is because cardiovascular risk factors, acting upon various aspects of Virchow's triad give rise to a hypercoagulable and atherogenic state. Before overt vascular disease manifests endothelial dysfunction occurs, which doesn't usually occur focally, although predominates around bifurcations and vascular tributaries. As there is no consensus however this study has made associations with both mean and maximum CCIMT. As there is no accepted normal reference range for CCIMT, associations were made using this as a continuous variable, rather than a dichotomous one.

In answer to the study hypothesis, ethnicity predicted both mean and maximum CCIMT, after adjusting for traditional cardiovascular risk factors. Black subjects had statistically significantly higher mean but not significantly higher maximum CCIMT than South Asians overall and in each sex. Previous studies that found CCIMT is significantly increased in Black populations after controlling for cardiovascular risk factors [D'Agostino et al. 1996; Markus et al. 2001; Kuller et al.1998] support the validity of this study's findings. This study also found that mean had significantly higher CCIMT on univariate analysis, which supports previous data [Allan et al. 1997] and while there was a trend towards men having higher

mean and maximum CCIMT on multivariate analysis this did not retain independence after adjusting for traditional cardiovascular risk factors.

CCIMT was associated with traditional risk factors in both ethnic groups although ethnic differences appeared to exist in both univariate and multivariate analyses. Amongst South Asian participants, hypertension was statistically significantly associated with increased both mean and maximum CCIMT on univariate analysis and SBP and PP were positively correlated with both mean and maximum CCIMT on univariate analysis, supporting previous literature [Zanchetti et al. 2001; Rossi et al. 2000; Pauletto et al. 1999]. As PP is a product of SBP and DBP its associations with CCIMT would be expected to be similar to SBP. The very strong correlation coefficients between these two measurements in South Asians and Blacks reflect this finding. SBP remained an independent predictor of both mean and maximum CCIMT in South Asians after adjusting for traditional cardiovascular risk factors.

Amongst Black participants, the presence of hypertension was not significantly associated with higher CCIMT in Blacks. However, SBP and PP were positively correlated with both mean and maximum CCIMT on univariate analysis. SBP did not remain an independent predictor of CCIMT after adjusting for traditional cardiovascular risk factors. This finding may either be due to the relatively small sample in the black cohort or may be due to the presence of co-existent cardiovascular risk factors of diabetes and smoking which would make it harder to find any independent effect of SBP after these were adjusted for.

Patients with diabetes have previously been found to have increased CCIMT when compared to non-diabetic populations [Poredos et al. 1997]. The presence of diabetes was associated with statistically significantly higher mean and maximum CCIMT in both South Asian and Black subjects on univariate analysis. However, when traditional risk factors were adjusted for it was no longer an independent predictor. As mentioned above, the presence of co-existent risk factors may have contributed to this finding. HbA1c was positively correlated with mean and maximum CCIMT in both ethnic groups on univariate analysis. Because of the low level of blood sampling in this study, particularly in the South Asian subjects, HbA1c was not brought forward into multivariate analyses. However one would expect it not to retain independence given that diabetes would have been adjusted for and this marker is inherently related to that risk factor.

Lifetime smoking has previously been found to be strongly related to CCIMT [Poredos et al. 2004]. Amongst the South Asian subjects, smoking resulted in higher mean and maximum CCIMT on univariate analysis and even after adjusting for traditional cardiovascular risk factors remained a significant independent predictor of mean CCIMT on multivariate analysis. Given that smoking leads to an atherogenic microenvironment, one would expect it to have a greater impact on mean CCIMT, which reflects an overall picture of atherogenesis rather than maximum or focal thickening. No relationship between smoking and CCIMT was found in the Black subjects on wither univariate or multivariate analysis, which suggests that smoking may have a greater impact on the development of vascular disease in South Asians, in spite of the prevalence of smoking being very low in this ethnic group. The findings may also reflect that other risk factors are more important in the progression of CCIMT in Blacks other than smoking.

With regards to dyslipidaemias, LDL-Cholesterol has previously been found to be strongly related to CCIMT [Poredos et al. 2004]. Amongst South Asians, total cholesterol and LDL-C were significantly correlated with mean and maximum CCIMT on univariate analysis. However due to the low levels of blood sampling these were not taken forward into the multivariate analysis due to the low level of statistical power.

This study supports previous associations between both asymptomatic and symptomatic PAD and CCIMT in other populations [Allan et al. 1997; McDermott et al. 2005]. On univariate analysis in both South Asian and Black subjects PAD was associated with higher mean and maximum CCIMT. This finding remained apparent on multivariate analysis even after adjusting for traditional cardiovascular risk factors, ethnicity and age. Given that PAD is in another vascular territory, i.e. the lower limbs, the finding of raised CCIMT in these subjects suggests that people with vascular disease in any vascular territory will have a greater atherogenic burden overall, which might explain the overlap found between PAD, CAD and CBVD [CAPRIE steering committee 1996; Pasternak et al. 2004].

Patients are usually started on antiplatelet and cholesterol lowering medication when they either have overt cardiovascular disease (e.g. CAD, PAD or CBVD) or are at high cardiovascular risk (e.g. Diabetics). One would expect that subjects taking these medications would therefore have greater mean and maximum CCIMT, If CCIMT is considered to be a marker of atherosclerosis and of cardiovascular risk [Baldessarre et al. 2007]. The finding of subjects on antiplatelets and cholesterol lowering medication having higher mean and maximum CCIMT on univariate analysis in both ethnic groups supports this.

Limitations

A number of different methods have been used to measure CCIMT. A semi-automated analysis system was used in this study to determine the mean and maximum CCIMT over a 1cm segment of the common carotid artery, whose central point is 2cm from the carotid bifurcation. This site was chosen as the intima-medial boundaries are better defined than in other segments [Folger et al. 1987; Zierler et al. 1987]. There is currently no standardisation in equipment and protocol for the measurement and recording of CCIMT. As such, caution must be taken when attempting to draw comparisons between results of different studies

Due to the cross-sectional design of this study, causal associations between socio-demographic variables and CCIMT could not be reported. Another limitation of this study is that of responder bias. Whilst all eligible South Asian and Black subjects were invited to participate in the E-ECHOES study, patients could respond via a free-phone telephone number or return a stamped addressed envelope. To minimise responder bias, especially from non-English speakers, subjects were telephoned and a verbal explanation was provided. Another possible bias was that consent for blood was low, especially amongst South Asian participants, which may have affected the strength of associations we found with CCIMT. However, the rate of blood sampling in this study was comparable to other national surveys [HSE 1999].

Another limitation of this community based study was that South Asian and Black participants were not matched on gender or on whether they were 1st or 2nd generation migrants. Whilst CCIMT comparisons were made by gender, differences in prevalence of risk factors and their associations with CCIMT may have been affected by differences in

gender proportions in each ethnic group. Subjects were invited to participate in this study based on their ethnicity. The significant difference in 1st generation migrants between the South Asian and Black groups may have had effects on the prevalence of cardiovascular risk factors and their associations with CCIMT.

3.3.7 Conclusion

In this study, Black ethnicity is related to greater mean and maximum CCIMT compared to south Asians, even after adjusting for traditional cardiovascular risk factors. There appear to be ethnic differences in the associations between CCIMT and traditional cardiovascular risk factors. The presence of PAD independently predicts mean and maximum CCIMT after adjusting for ethnicity, age and traditional cardiovascular risk factors.

3.4 Haemostatic Cardiovascular Risk Factors, Common Carotid Intima-Medial Thickness and Peripheral Arterial Disease in South Asians and Blacks

3.4.1 Abstract

Objectives: To determine whether ethnic differences exist in inflammatory (interleukin-6 and C-reactive protein) and hemostatic biomarkers (platelets, soluble P-selectin [sP-sel], von Willebrand factor [VWF], and fibrin D-dimer) between South Asian (people originating from India, Pakistan, and Bangladesh) and Black (Black Caribbean and Black African) groups, the two largest minority ethnic groups in the UK. To determine associations between these biomarkers and common carotid intima–media thickness (CCIMT) and peripheral arterial disease (PAD).

Methods: 572 subjects (356 South Asian and 216 Black) aged ≥ 45 years were recruited as a sub-study to a community screening project, the Ethnic-Echocardiographic Heart of England Screening (E-ECHOES) study. All subjects completed an interviewer-led questionnaire, anthropometric measurements were taken, and blood sampling was performed if consent was granted. ABPI was calculated, and the CCIMT was measured. PAD was defined as ABPI < 0.9. ELISA was used to quantify inflammatory and hemostatic biomarkers.

Results: The incidence of hypertension (>70%) and diabetes (>27%) was high, but non-significantly different between the two ethnic groups. South Asians had higher platelet count and sP-sel levels than Blacks ($p<0.0001$ for both) on univariate analysis, despite there being no significant difference in antiplatelet medication. Blacks had higher D-dimer levels ($p=0.0052$) on univariate analysis. Among South Asians, VWF correlated with ABPI ($p=0.047$) and mean ($p=0.002$) and maximum CCIMT ($p=0.011$) on univariate analysis, and

remained an independent predictor of mean and maximum CCIMT on multivariate analysis with traditional cardiovascular risk factors ($p=0.034$ and $p=0.046$, respectively). In Blacks, D-dimer levels were higher in PAD than in normal ABPI participants ($p=0.04$), and was associated with ABPI in both univariate analysis ($p=0.014$) and multivariate analysis ($p<0.0001$) with traditional cardiovascular risk factors.

Conclusion: Ethnic differences are evident in inflammatory and hemostatic factors, as well as in their associations with CCIMT and PAD. These may reflect differences in cardiovascular risk factors or pathophysiologic processes that characterize each ethnic group.

3.4.2 Introduction

Peripheral arterial disease (PAD) is an important global healthcare problem associated with considerable morbidity and mortality [Norgren et al. 2007]. This disease is an important manifestation of atherosclerosis and the pathophysiological processes involved in its development, progression and complications are endothelial dysfunction/damage leading to atherothrombosis and thromboembolism.

Given improvements in biochemical techniques, there has been an increased interest in the prognostic value of biomarkers in the development of PAD and its symptomatic complications. For example Von Willebrand Factor (VWF), an established index of endothelial dysfunction/ damage has been shown to be elevated in PAD [Seigneur et al. 1995; Lee et al. 1995; Cassar et al. 2003; Cassar et al 2005]. Platelet activation is a crucial component of thrombogenesis and may be quantified by measurement of soluble P-selectin (sP-sel). sP-sel has been associated with prevalent PAD [Ridker et al. 2001] and platelet concentration and sP-sel have been reported to independently predict severity of PAD [Tan et al. 2005].

Another biomarker, fibrin D-dimer, is a fibrin degradation product that marks on-going fibrin turnover and reflects a hypercoagulable state [Matsuo et al. 2000]. D-dimer correlates with both symptomatic and asymptomatic PAD and also with disease severity [Lee et al. 1995, Tzoulaki et al. 2007] and functional impairment [McDermott et al. 2003]. Inflammation also plays a major role in all stages of atherosclerotic vascular disease, and

Interleukin-6 (IL-6) and CRP are associated with PAD development, progression and its severity [Ridker et al. 2001; Tzoulaki et al. 2007; Aboyans et al. 2006; Tzoulaki et al. 2005].

Abnormalities in markers of thrombogenesis (novel risk factors) appear to be related to, or even additive to, traditional cardiovascular risk factors [Ridker et al. 1997; Khalegi et al. 2009] in the initiation and acceleration of atherogenesis. There is also evidence of the prognostic value for these biomarkers of thrombogenesis, which suggests that they are not merely consequences of atherothrombotic diseases, such as PAD, but may actively contribute to the pathogenesis of vascular disease and its complications [Lip & Blann 2004]. Even after adjusting for traditional risk factors some biomarkers still remain significantly associated with PAD [Khawaja et al. 2007; Allison et al. 2006]. However, despite ethnic differences having been documented in PAD prevalence groups [Table 1.1] [Premalatha et al. 2000, Criqui et al.2005; UK Prospective Diabetes Study Group 1994; Mohan et al. 1995; Chaturvedi et al.2007; Collins et al. 2005; McDermott et al.2005], much of the research on PAD pathophysiology has been carried out on predominantly White European populations, and limited research has been directed on these biomarkers in relation to the ethnic susceptibility to PAD [Allison et al. 2006].

The aim of this study was to investigate whether markers of endothelium damage/ dysfunction (VWF), thrombogenesis (Platelets, sP-sel, D-dimer) and inflammation (IL-6, CRP) are associated with common carotid intima media thickness (CCIMT) and PAD in South Asians (that are, people originating from India, Pakistan and Bangladesh) and Blacks (Black Caribbean and Black African) as these are the most prevalent minority ethnic groups in the UK.

3.4.3 Hypothesis to be tested

There will be ethnic differences in associations between circulating markers of inflammation, haemostasis and thrombosis (CRP, IL-6, D-dimer, sP-sel, VWF) and CCIMT and PAD in South Asians and Blacks.

3.4.4 Patients & Methods

Study Design &Recruitment

Details of the study design and recruitment into the E-ECHOES study and into this sub-study are described in detail in Chapter 2.4.1.2 on page 82.

Clinical Assessment

The clinical assessment performed in described in detail in chapter 2.4.1.3 on page 86.

Common Carotid Artery Intima Medial Thickness (CCIMT) Assessment

CCIMT assessment is described in chapter 2.4.1.4 on page 88.

PAD Assessment

A description of how PAD was assessed is found in chapter 2.4.1.6 on page 92.

Laboratory

Venous blood was taken atraumatically from the antecubital fossa by the student for routine laboratory tests, including full blood count, Urea & electrolytes and D-dimer, as well as for research indices. Blood was stored on ice for a maximum of 2 hours before being spun down at 1500 x g; the supernatant was then frozen at -70ºC for future batch analysis. Samples were tested for novel risk factors using commercially available ELISA kits: VWF (Dako, Ely, UK), IL-6 (R&D systems reagents, Abingdon, UK), CRP (Biokit, SA, Barcelona, Spain), sP-sel (R&D systems, Abingdon, UK). D-dimer was measured with a Cobas Integra 400 plus auto-analyser (Roche Diagnostics, UK). Intra-assay and inter-assay variances of all assays

were <5% and <10% respectively. Lower limits of detection were IL-6: 9.375 pg/ml, sP-sel: 0.125 ng/ml, VWF: 25 iu/dl, CRP: 0.71 mg/l. Consent for blood sampling was obtained in 269 out of 572 (47%) of this sub-study to E-ECHOES (143 out of 356 (40.2%) South Asians and 126 out of 216 (58.3%) Blacks).

Power calculation

For correlations between haemostatic risk factors and CCIMT and ABPI a power calculation was set to detect a correlation coefficient of r>0.25, for alpha p<0.05 and 1-ß >0.8. This required 123 subjects to minimise the risks of type 1 and type 2 errors. In the analysis of ethnic differences on haemostatic risk factor expression [Table 3.25] a sample size of 250 brings the alpha p<0.05 and 1-ß p >0.8 to determine a real difference of 0.22 of a standard deviation. In the analysis of impact of gender on expression of haemostatic risk factors [Tables 3.26.1 & 3.26.2] a sample size of 100 for each gender brings the alpha p<0.05 and 1-ß p>0.8 to determine a real difference of 0.35 of a standard deviation.

Statistical Analysis

Statistical analysis was undertaken using Minitab version 15 (State Coll, PA, USA). Data were summarised using mean, median, standard deviation and inter-quartile range for continuous parameters. Student's t-test and Mann-Whitney U test were used for differences in continuous variables and Chi-squared test for categorical data. Spearman rank correlation coefficient was calculated to test the association between the ABPI and a number of risk factors. Based on previous reports on the dangers of multiple significance testing in cross-sectional studies (described in chapter 2.4.1.10 on page 98), a Bonferroni correction was not

performed and a p-value <0.05 was deemed significant. In an attempt to see is any of the research indices in this study were independently predictive of CCIMT and ABPI, they were all entered into multivariate analyses adjusting for traditional cardiovascular risk factors.

3.4.5 Results

Between March 2008 and February 2009, 574 subjects (358 South Asians and 216 Blacks) were recruited, aged between 45 and 100 years; 2 subjects with ABPI >1.39 were excluded, bringing the total analysed to 356 South Asians and 216 Black subjects [Table 3.24]. In both groups the incidence of diabetes and hypertension was high but equivalent [Table 3.24]. Mean SBP was also high in both South Asians (142 (20) mmHg) and Blacks (145 (18) mmHg). South Asians had a greater proportion of men, ($p=0.005$), 1st generation migrants ($p<0.0001$), higher illiteracy ($p<0.0001$), higher triglycerides ($p<0.0001$) and HbA1c ($p=0.0007$) [Table 3.24] and lower BMI ($p=0.001$) than Blacks. South Asians also had a greater proportion of never smokers than Blacks ($p<0.0001$).

The ethnic breakdown of the South Asian group was: 40.4% Pakistani, 38.9% Indian, 16.9% Bangladeshi and 2.8% East African Asian, broadly representative of the distribution of these ethnic groups within Birmingham [National Statistics Online]. The South Asian group was analysed as one heterogeneous group in order to improve the statistical power of any findings.

Table 3.24: Clinical and Demographic characteristics of South Asian and Black subjects

Variable	South Asian (n=356) (SD) [IQR]	Black (n=216) (SD) [IQR]	p-value
Demographic Details			
Age (years)	61 (11)	63 (11)	0.108
Male (%)	58.7	46.8	0.005
1st generation migrants (%)	98.6	86.1	<0.0001
Illiteracy (%)	30.1	0	<0.0001
BMI	28 (4.9)	29.5 (5.8)	0.001
Waist circumference (cm)	98.4 (13.1)	97.6 (12.8)	0.452
SBP (mmHg)	142 (20)	145 (18)	0.085
DBP (mmHg)	82 (11)	83 (10)	0.43
PP (mmHg)	60 (16)	62 (17)	0.137
Resting Pulse rate (bpm)	80 (13)	78 (13)	0.042
Cardiovascular Risk Factors			
Diabetes (%)	30.6	27.3	0.4
Hypertension (%)	70.8	75.5	0.224
Never Smoker (%)	71.3	51.8	<0.0001
Ex-smoker (%)	16.7	26.1	0.011
Current Smoker (%)	12	22.1	0.003
Vascular disease states			
CAD (%)	12.9	6.5	0.01
CBVD (%)	3.7	4.8	0.5
PAD (%)	13.2	10.2	0.283
Vascular measurements			
Lowest side ABPI	1.02 (0.1)	1.04 (0.1)	0.11
Mean CCIMT (mm)	0.61 (0.13)[€]	0.64 (0.14)[Y]	0.022
Maximum CCIMT (mm)	0.73 (0.16)[€]	0.75 (0.16) [Y]	0.113
Vascular disease treatment			
Antiplatelet usage (%)	34.8	33.8	0.8
Cholesterol lowering medication (%)	48	42.6	0.2
Laboratory measurements			
Triglycerides (mmol/l)	2.0 [1.3-2.7] [¥]	1.36 [0.7-2.2] [κ]	<0.0001
LDL-C (mmol/l)	2.3 [1.8-2.9] [¥]	2.52 [1.8-3.1] [κ]	0.297
HDL-C (mmol/l)	1.1 [0.8-1.2] [¥]	1.32 [1.1-1.6] [κ]	<0.0001
Total Cholesterol (mmol/l)	4.4 [3.8-5.2] [¥]	4.63 [3.7-5.3] [κ]	0.295
HbA1c (%)	6.7 (1.3) [¥]	6.1 [5.7-6.6][κ]	0.0007

Research Indices			
sP-sel (ng/ml)	32.9 [26.2 - 41.6]$^{\beta}$	25.6 [20.4 - 32.9]$^{\alpha}$	<0.0001
Platelets (x10^{3}cell/µl)	282.5 (74)$^{\beta}$	251.7 (74)$^{\alpha}$	<0.0001
D-dimer (µg/ml)	0.26 [0.15 - 0.49]$^{\beta}$	0.36 [0.19 - 0.69]$^{\alpha}$	<0.0052
VWF (iu/dl)	75.3 (13.7)$^{\beta}$	78.2 (17)$^{\alpha}$	0.132
CRP (mg/l)	1.95 [0.76 - 5.03]$^{\beta}$	1.71 [0.55 - 3.92]$^{\alpha}$	0.179
IL-6 (pg/ml)	42.5 [16.3-230.4]$^{\beta}$	47.4 [19.4-207.4]$^{\alpha}$	0.635

Data are mean (SD) or median [IQR]. IQR: Inter-quartile range; SD: standard deviation. $^{¥}$: n=143; $^{\kappa}$: n=126; $^{€}$: n= 293; ^{Ý}: n= 199; $^{\beta}$: n=134; $^{\alpha}$: n=129. SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA1c: glycosylated haemoglobin, ABPI: ankle brachial pressure index; CAD: coronary artery disease; CBVD: cerebrovascular disease; PAD: peripheral arterial disease; IC: intermittent claudication; sP-sel: soluble P-selectin; VWF: Von Willebrand factor; CRP: C-reactive protein; IL-6: interleukin-6.

Ethnic differences in indices of haemostasis and inflammation

Overall South Asians had statistically significantly higher platelets ($p<0.0001$) and sP-sel ($p<0.0001$) despite no differences in antiplatelet usage and Blacks appeared to have statistically significantly higher levels of D-dimer ($p=0.0052$) [Table 3.24]. Amongst women, South Asians had statistically significantly higher sP-sel ($p=0.0015$) and platelets ($p=0.03$) and a lower D-dimer ($p=0.0172$) and VWF ($p=0.017$) than Blacks [Table 3.25]. The findings in men supported those found in the female subjects in that South Asians had statistically significantly higher sP-sel ($p=0.0001$) and platelets ($p=0.046$) than Black subjects. South Asian men also had statistically significantly higher CRP ($p=0.0382$) than Blacks [Table 3.25]. No differences were found in IL-6 between the ethnic groups overall or by sex.

When comparing the markers of haemostasis and inflammation between subjects with and without PAD this study found no statistically significant differences and so associations were investigated using ABPI as a continuous variable.

Table 3.25: Ethnic differences in expression of indices of Haemostasis and Inflammation by sex

3.25.1: Male subjects:

Variable	South Asian (n=84) (SD) [IQR]	Black (n=54) (SD) [IQR]	p-value
sP-sel (ng/ml)	33.8 [28.4- 42.1]	26.9 [21.2- 35.2]	0.0001
Platelets (x10^{3}cell/µl)	272.4 (68.8)	251.7 (74.1)	0.046
D-dimer (µg/ml)	0.26 [0.15- 0.49]	0.33 [0.19-0.56]	0.229
VWF (iu/dl)	76.7 (14.9)	76.3 (13.8)	0.874
CRP (mg/l)	1.7 [0.71- 5.3]	1.15 [0.5- 2.8]	0.0382
IL-6 (pg/ml)	28.7 [13.3-206.6]	74.4 [22-236]	0.124

3.25.2: Female subjects:

Variable	South Asian (n= 50) (SD) [IQR]	Black (n= 75) (SD) [IQR]	p-value
sP-sel (ng/ml)	31.4 [23.5- 40.5]	24.9 [19.3- 31.8]	0.0015
Platelets (x10^{3}cell/µl)	299.7 (81.3)	267.2 (73.8)	0.031
D-dimer (µg/ml)	0.24 [0.15- 0.46]	0.37 [0.24- 0.82]	0.0172
VWF (iu/dl)	73.1 (11.4)	79.5 (18.9)	0.017
CRP (mg/l)	2.69 [0.82-4.5]	2.05 [0.72-5.35]	0.754
IL-6 (pg/ml)	65.2 [23.7-260.3]	41.9 [16.7-153.9]	0.250

sP-sel: soluble P-selectin; IL-6: Interleukin-6; VWF: Von Willebrand Factor; CRP: C-reactive protein; SD: standard deviation; IQR: inter-quartile range.

Associations between indices of haemostasis and inflammation with ABPI, mean and maximum CCIMT

South Asians:

Based on my power calculation to minimise the risks of types 1 and 2 errors, the only significantly powered association in South Asians on univariate analysis was between VWF and mean CCIMT (r=0.283; p=0.002) [Table 3.22]. There was a weak, but not significantly powered association between VWF and maximum CCIMT (p=0.011) and ABPI (p=0.047) on univariate analysis. Another weak association was found between D-dimer and mean CCIMT (p=0.043) on univariate analysis.

I looked for univariate associations between indices of haemostasis and inflammation and traditional cardiovascular risk factors and in South Asians found VWF and D-dimer both correlated with age (r=0.347; p<0.0001 and r=0.458; p<0.0001 respectively). There was also a weak correlation between platelets and LDL-C (r=0.251; p=0.008). However these results were not significantly powered.

I wanted to see if any of the indices of haemostasis and inflammation was an independent predictor of mean CCIMT (Model 1), maximum CCIMT (Model 2) and ABPI (Model 3) when adjusting for traditional cardiovascular risk factors on multivariate analysis. Of the research indices investigated, VWF was an independent predictor of mean (p=0.034; R^2 for model=19.3%) and maximum (p=0.046; R^2=18.4%) CCIMT and D-dimer was an independent predictor of mean CCIMT (p=0.036; R^2=18.5%). None of the research indices was an independent predictor of ABPI in the South Asian subjects.

Table 3.26.1: Univariate correlations between novel cardiovascular risk factors, ABPI, mean and maximum CCIMT

3.26.1: South Asians

Variable	ABPI r-value	ABPI p-value	Mean CCIMT r-value	Mean CCIMT p-value	Maximum CCIMT r-value	Maximum CCIMT p-value
sP-sel (ng/ml)	0.036	0.664	0.038	0.678	0.098	0.283
Platelets ($x10^3$cell/µl)	0.015	0.872	0.020	0.837	0.047	0.629
D-dimer (µg/ml)	-0.005	0.95	0.186	0.043	0.108	0.36
VWF (iu/dl)	-0.168	0.047	0.283*	0.002	0.232	0.011
CRP (mg/l)	0.063	0.461	0.122	0.192	0.086	0.36
IL-6 (pg/ml)	-0.106	0.323	0.021	0.861	-0.013	0.910

3.26.2: Blacks

Variable	ABPI r-value	ABPI p-value	Mean CCIMT r-value	Mean CCIMT p-value	Maximum CCIMT r-value	Maximum CCIMT p-value
sP-sel (ng/ml)	-0.033	0.709	0.148	0.104	0.21	0.02
Platelets ($x10^3$cell/µl)	0.102	0.254	-0.201	0.03	-0.201	0.03
D-dimer (µg/ml)	-0.214	0.014	-0.067	0.464	-0.032	0.727
VWF (iu/dl)	-0.140	0.109	0.027	0.767	0.01	0.909
CRP (mg/l)	-0.0003	0.974	0.156	0.095	0.159	0.09
IL-6 (pg/ml)	-0.07	0.537	0.025	0.838	0.09	0.454

sP-sel: soluble P-selectin; IL-6: interleukin-6; VWF: Von Willebrand factor; CRP: C-reactive protein; SD: standard deviation; IQR: inter-quartile range; ABPI: ankle brachial pressure index; CCIMT: common carotid intima media thickness; *: sufficiently powered result

Blacks:

Amongst Black subjects this study observed no sufficiently powered significant associations between research indices, ABPI and mean and maximum CCIMT. D-dimer was weakly associated with ABPI (p=0.014) [Table 3.22] and platelet count was weakly associated with both mean and maximum CCIMT (p=0.03 for both) and sP-sel was weakly associated with maximum CCIMT (p=0.02) [Table 3.22].

Univariate associations between traditional cardiovascular risk factors and research indices this study observed were between platelet count and age (r=-0.259; p=0.003) and D-dimer and mean SBP and age (r=0.25; p=0.004 and r=0.404; p<0.0001 respectively).

On multivariate analysis, adjusting for traditional cardiovascular risk factors, of the research indices studied, only D-dimer remained an independent predictor of ABPI (p<0.0001; R^2 for model=21.8%). None of the research indices was an independent predictor of either mean or maximum CCIMT on multivariate analysis after adjusting for traditional cardiovascular risk factors.

3.4.6 Discussion

This is the first community based study of ethnic associations between circulating inflammatory and haemostatic biomarkers, CCIMT and ABPI in the UK. This study reports ethnic differences in biomarker profile and report different associations between biomarkers, CCIMT and ABPI in South Asians and Blacks. Notably Blacks had significantly higher D-dimer than South Asians overall and in women, whilst South Asians had higher sP-sel and platelet concentrations overall and in each sex, despite equivalent antiplatelet medication use between the two ethnic groups. Of the haemostatic biomarkers, D-dimer was the only one which significantly differed between the PAD and normal ABPI cohorts in Blacks, supporting findings reported in predominantly White European populations [Lee et al. 1995; Tzoulaki et al. 2007].

Differences in prevalence of cardiovascular diseases are known to exist between ethnic groups. In particular, South Asians and Blacks have a higher cardiovascular disease burden when compared to the general population [Gill et al. 2007]. This increased susceptibility is not completely explained by prevalence if traditional cardiovascular risk factors. Indeed, novel risk factors may play some role at least in the development of vascular diseases, such as PAD, possibly in the ethnic susceptibility to cardiovascular disease [Allison et al. 2006; Khalegi et al. 2009].

D-dimer is a sensitive marker of thrombosis formation and subsequent fibrinolysis and higher D-dimer maybe indicative of a pro-coagulant state favouring progression of atherosclerosis and its thrombotic complications. This study's findings of higher D-dimer

support those in the US comparing African Americans to other ethnic groups [Lutsey et al. 2006]. Even though African Americans and UK Blacks differ with regards to their cardiovascular risk and healthcare access [Forouhi et al. 2006], this study suggests Black ethnicity may be associated with a greater predisposition to a hypercoagulable and prothrombotic state than other ethnic groups, which may in part explain the increased prevalence of PAD and thrombotic complications in this ethnic group [Norgren et al. 2007].

Whilst equivalent levels of VWF were found in South Asians and Blacks overall; women in the latter group had higher levels than the former. This supports findings of a recent US study investigating markers of cardiovascular disease also reported VWF to be independently associated with being an African American female [Kim et al. 2010]. Of the haemostatic biomarkers, VWF was also independently associated with mean and maximum CCIMT on linear regression in South Asians. VWF is considered a well-validated plasma marker for the measurement of endothelial dysfunction/ damage in atherosclerosis [Zanetta et al. 2000]. This study's findings may reflect greater endothelial damage/dysfunction and possibly higher atherosclerotic load, but not overt disease, in South Asians.

There is evidence to suggest that there are regional differences in atherosclerotic risk susceptibility to specific haemostatic biomarkers [Chaturvedi et al. 2007]. It is also possible that different pathophysiological processes are more predominant in different ethnic groups, which may contribute to the susceptibility of disease in different vascular territories. If this is the case, VWF may play a role in the susceptibility of South Asians to atherosclerotic disease of carotid and coronary arteries. D-dimer on the other hand, reflecting the hypercoagulable and pro-thrombotic state found in PAD [Makin et al. 2002; Chung et al.

2003/2004; Lowe et al. 2003/2004], and its thrombotic complications, may in part explain the apparent higher susceptibility to PAD with Black ethnicity [Norgren et al. 2007].

VWF has previously been associated with traditional cardiovascular risk factors [Blann et al. 1993a; Blann et al. 1993b, Spencer et al. 2002] and PAD [Seigneur et al. 1995; Lee et al. 1995; Cassar et al. 2003; Cassar et al. 2005]. This study found VWF to be associated with age but not with LDL-C, diabetes, hypertension or smoking in South Asians. D-dimer was associated with age only in South Asians and SBP and age in Blacks, supporting literature in other populations [Lee et al. 1995; Khalegi et al. 2009]. Other associations did not reach sufficient statistical power.

It was surprising to find no associations between inflammatory markers and PAD in this study. Likewise the lack of an association between CRP and IL-6 with ABPI and mean and maximum CCIMT contrasts with published findings of their associations in White populations [Ridker et al. 2001; Tzoulaki et al. 2007; Aboyans et al. 2006; Tzoulaki et al. 2005]. This may reflect different pathophysiological processes predominating in different ethnic groups, as discussed above or could be a result of small numbers of subjects in this study.

Limitations

This study is limited by its cross-sectional design and the low numbers of participants in some of the subgroups studied. Participant numbers were small, especially those with PAD, which may have affected some associations between haemostatic factors, CCIMT and ABPI. Consent for blood was low, especially amongst South Asians, but comparable to those found

in national surveys [Health Survey for England 1999]. The low numbers of participants and low blood sampling may have contributed to the lack of sufficient power in some of the results, which may be subject to types 1 and 2 errors. This study population consisted of predominantly 1st generation UK migrants. These are less likely to have adopted a Western lifestyle than subsequent generations, which in itself may enhance cardiovascular risk and influence the results in this chapter. However differences in the proportion of 1st generation migrants between South Asians and African Caribbeans may have contributed to differences in haemostatic risk factors in these populations.

Another possible limitation of this study is that of responder bias. Whilst all eligible subjects were invited to take part in the E-ECHOES study, patients responded via a free-phone telephone number or return a stamped addressed envelope. To minimise response bias especially from non-English speakers, subjects were also telephoned and a verbal explanation was provided. It is also possible that eligible subjects with existing cardiovascular disease were more likely to participate in a cardiovascular screening study than those without, which may have affected our results. This study population had higher incident diabetes and hypertension than previous studies [Chaturvedi et al. 1994; Diabetes in the UK 2004], which may have contributed to the associations with haemostatic risk factors which we found. However the incidence of these risk factors was equivalent in South Asian and Black subjects. Likewise significant differences in other cardiovascular risk factors, notably dyslipidaemias, BMI, smoking and incident CAD may have played a role in contributing to the differences in haemostatic risk factors between the South Asian and Black groups. It is difficult to know whether ethnicity per se or differences in cardiovascular risk factors between the 2 ethnic groups played the greater role in this study's findings.

Nonetheless, this study still represents the first epidemiological-based study of pathophysiological biomarkers in ethnic minority groups in the UK community setting.

3.4.7 Conclusion

Ethnic differences are evident in inflammatory and haemostatic factors, and their associations with CCIMT and PAD. Whilst this may reflect differences in cardiovascular risk factors, this may reflect different pathophysiological processes predominating in different ethnic groups.

CHAPTER 4

HOSPITAL BASED STUDY

4.1 Ethnic Differences in Circulating Markers of Angiogenesis and their Association with Cardiovascular Risk Factors and Cardiovascular Disease

4.1.1 Abstract

Objectives: To determine (a) whether ethnic/racial differences exist in circulating markers of angiogenesis (Angiopoietin-1 (Ang-1), Angiopoietin-2 (Ang-2), soluble Tie-2 receptor (sTie2) and Angiogenin) between South Asian (from India, Pakistan and Bangladesh); Black African-Caribbean and White ethnic groups, and (b) associations between these markers in stable cardiovascular disease (CVD) and cardiovascular risk factors.

Methods: 243 (82 South Asian, 84 Black and 77 White) subjects were recruited into this study; Symptomatic and clinically confirmed CVD (n=108), risk factor controls (with ≥1 cardiovascular risk factor, e.g. smoking, diabetes mellitus, dyslipidaemia, hypertension) and with ankle brachial pressure index >1) (n=64) and healthy controls free of CVD and risk factors (n=56). Angiogenic markers were measured by enzyme linked immunoassay.

Results: There were no statistically significant differences in any of the research indices when comparing all subjects in the 3 ethnic groups. In healthy controls, angiogenin was higher in South Asian and Black subjects, compared to Whites (p=0.05). sTie2 correlated inversely with angiogenin (p=0.001), was higher in women (p=0.029) and was lower in smokers (p=0.007). Overall, age (p=0.001) was the only independent factor associated with

angiopoietin-1. Angiogenin (p=0.01) and SBP (p=0.014) were both independently higher in the Black group compared to the White group.

Conclusions: Ethnic, racial, and demographic differences are evident in certain circulating markers of angiogenesis. With the exception of an effect of smoking on sTie2, these differences are not influenced by the presence of other risk factors, nor the presence of stable cardiovascular disease.

4.1.2 Introduction

Cardiovascular disease, manifesting as peripheral arterial disease, coronary artery disease or cerebrovascular disease, is an important consequence of atherosclerosis. Angiogenesis, the formation of new blood vessels, is evident in these conditions and is closely related to atherogenesis, thrombogenesis and the development of intra-plaque vasa vasorum [Sluimer & Daemen 2009; Post et al. 2004]. Furthermore, angiogenesis is a feature of disease development and progression [Moreno et al. 2004; Kolodgie et al. 2003], and is especially frequent in advanced cardiovascular disease and may characterise atherosclerotic lesions at high risk of haemorrhage or rupture [Kolodgie et al. 2003; McCarthy et al. 1999; Mofidi et al. 2001]. Vascular endothelial growth factor (VEGF), acting on the endothelium, is one of the major growth factors driving angiogenesis, and raised plasma levels in diabetes, peripheral artery disease and coronary artery disease is evidence of this increased angiogenic activity [Blann et al. 2002].

In addition to VEGF, the angiopoietins (Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) are additional angiogenic growth factors that are specific for endothelium. Ang-1 and Ang-2 play modulatory roles by binding a common receptor, the endothelial cell-specific tyrosine kinase receptor Tie-2 [Chong et al. 2004; Van der Heijen et al. 2010], a soluble form of which (i.e. sTie2) can be detected in plasma. Together, the angiopoietins regulate endothelial cell differentiation: Ang-1 accelerates the maturation of the blood vessel, while Ang-2, an antagonist of Ang-1, destabilises the vessel and degrades the basal lamina [Felmelden et al. 2003; Stetler-Stevonson et al. 1999]. Plasma levels of Ang-1 and Ang-2 levels are abnormal in, and are associated with, cardiovascular risk in patients with cardiovascular disease

[Findley et al. 2008] and also in other manifestations of atherosclerosis, including heart failure, acute coronary syndromes and hypertension [Chong et al. 2004; Felmelden et al. 2003a; Felmdelen et al. 2003b; Nadar et al. 2005; Lim et al. 2004; Lee et al. 2004].

Angiogenin is a small polypeptide implicated in angiogenesis normally found in the vasculature, but also in some physiological and pathological conditions, including peripheral and coronary atherosclerosis [Bond & Valee 1990; Burgmann et al. 1996; Tello-Montoliu et al. 2006]. Angiogenin levels have been found to be higher in those with severe peripheral atherosclerosis, compared to mild and moderate disease [Burgmann et al. 1996], and raised levels in acute coronary syndromes predict a poor outcome at six months [Tello-Montoliu et al. 2006]. Thus, angiogenin could potentially be an indicator of endothelial activity related to progression of vascular disease.

Given improvements in biochemical techniques there has been an increasing interest in value of biomarkers related to the development of atherosclerosis and its symptomatic complications. Despite ethnic and racial differences having been documented in the prevalence of atherosclerosis, much of the research on pathophysiology has been carried out on predominantly White populations [Burgmann et al. 1996; Tello-Montoliu et al. 2006] and limited research has been directed on these biomarkers in relation to ethnic and/or racial susceptibility to this disease [Allison et al. 2006].

4.1.3 Hypotheses to be tested

Ethnic differences will exist in plasma expression of angiogenic markers (Ang-1, Ang-2, Angiogenin, sTie2). Ethnic differences will also exist in the association of angiogenic markers with traditional cardiovascular risk factors and cardiovascular disease. Furthermore this study also tested the hypothesis that Ang-1, Ang-2, sTie2 and angiogenin levels would be higher in those with at least one cardiovascular risk factor compared to healthy controls, with a further increase in those with clear cardiovascular disease.

4.1.4 Patients & Methods

Study Design & Recruitment

Participants were recruited from among those attending cardiovascular disease outpatient clinics (cardiology and peripheral artery disease) and from hypertension clinics. Recruitment to this study is described in detail in chapter 4.2.2.2 on page 100.

Exclusion criteria for all subjects were infectious diseases, rheumatoid arthritis and other chronic inflammatory disorders, sepsis, malignancy, haemodynamically significant valvular heart disease, atrial fibrillation, renal failure, immunity-modulating drugs and hormone replacement therapy, recurrent venous thromboembolism, congestive cardiac failure, multiple sclerosis and anaemia. The study was approved by Birmingham East, North & Solihull Local Research Ethnics Committee and written informed consent was obtained from all study participants.

Clinical Assessment

The clinical assessments undertaken for this study are described in detail in chapter 4.2.2.3 on page 103.

Laboratory

Venous blood was taken from the antecubital fossa into sodium citrate. Blood was stored on ice for a maximum of 2 hours before being centrifuged at 1500 x g; the plasma supernatant was then frozen at -70ºC for future batch analysis. Samples were tested for angiogenic markers using commercially available ELISA kits (R&D systems, Abingdon. UK). Intra-

assay and inter-assay variances of all assays were <5% and <10% respectively. Lower limits of detection were 3.8ng/ml for Ang-1, 0.1 ng/ml Ang-2, 0.1 ng/ml angiogenin and 1.0 ng/mL for sTie2. All patients consented for blood to be collected for use in this study.

Sample size justification and statistical analysis

Data are presented as mean with standard deviation (SD) or median with inter-quartile range (IQR) for continuous parameters, as distribution defined. Chi-squared test was used for categorical data. One way ANOVA or the Kruskal-Wallis test was used to assess differences between three groups (South Asian, Black, White, and Healthy controls, cardiovascular risk factor, cardiovascular disease) for continuous variables, with Tukey's post-hoc analysis performed for inter-group differences. Determinants of the four research indices on multivariate analysis were sought using a general linear model, inputting ethnic group and sex as nominal categories, disease progression state as an ordinal category. When necessary, data was logged for normality. A p-value <0.02 was deemed significant. Two-sided Spearman correlations were sought with a 1-beta power of 0.85: a correlation coefficient of >0.4 was considered large enough to minimise false positives [Machin & Campbell 1987]. This study hypothesised a 25% - 50% difference in one of the research indices in one of the three nominal groups of SA, AC and White, and of a similar increase across the spectrum of health (modelling 100 [90-140] units/mL), to risk factors (125 [115-165] units/mL) to cardiovascular disease (150 [149-190] units/mL). To achieve this at $p<0.001$, a sample size of 50 per group was required. However, with four research indices, and expecting greater heterogeneity in the CVD group, this study recruited in excess in each group for extra statistical confidence. Statistical analyses were carried out using Minitab version 15 (State Coll, PA, USA).

4.1.5 Results

243 subjects with a mean age of 64.3 (SD 11.9) years were recruited. Diabetes was present in 25.9% and 55.1% were male. Breakdown by age, sex, blood pressure, body mass index, clinical grouping, risk factors, cardiovascular territory and use of aspirin according to racial/ethnic group is presented in table 4.1.

Table 4.1: Clinical and demographic details of Hospital Study Participants

Variable	South Asian (n=82) (SD)	Black (n=84) (SD)	White (n=77) (SD)	P-value
Demographic details				
Age (years)	62 (10)	66 (13)	63 (11)	0.146
Male sex (%)	60.7	48.3	54.4	0.254
SBP (mmHg)	142 (27)	144 (21)	134 (18)	0.024
DBP (mmHg)	81 (13)	80 (12)	76 (9)	0.020
BMI (kg/m2)	26.7 (4.4)	28.9 (5.5)	27.8 (5.2)	0.018
Clinical grouping				
Healthy Controls (%)	21.9	22.6	26.0	
Risk factor controls (%)	18.3	33.3	32.5	0.102
Cardiovascular disease (%)	59.7	44.0	41.4	
Cardiovascular risk factors				
Current smoking (%)	12.2	21.4	24.7	0.115
Diabetes (%)	31.7	30.9	14.3	0.019
Hypertension (%)	63.4	66.7	57.1	0.450
Dyslipidaemia (%)	55.1	46.1	49.4	0.879
Cardiovascular disease territory				
PAD (%)	30.5	34.5	36.4	
CAD (%)	35.4	14.3	20.8	0.154
CBVD (%)	11.0	7.1	6.5	
Use of antiplatelets (%)	50.0	46.6	49.3	0.887

Data are % or mean (standard deviation); SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; PAD: peripheral arterial disease; CAD: coronary artery disease; CBVD: cerebrovascular disease. Age, SBP, DBP, BMI analysed by ANOVA, all others by chi-squared

Subjects in the three ethnic groups (South Asian, Black, White) were matched for age and sex and were also matched for the proportion of healthy volunteers, subjects with actual cardiovascular risk factors (smoking, hypertension, dyslipidaemia, & diabetes) (Risk factor controls) and those who had CVD. The three ethnic groups were also matched for the proportion of subjects with disease in each cardiovascular disease territory and matched for use of aspirin. SBP and DBP were lower in Whites compared to the other two groups, whilst BMI was lower in South Asian compared to the Black group. There were fewer diabetics in the White group compared to the other two groups. The risk factor group consisted of 42 people with a single risk factor, 25 with two risk factors and one person with all four risk factors. Of those with cardiovascular disease, 80 had disease in one territory, 33 had disease in two and 4 patients had disease in all three.

Univariate analysis of the angiogenic markers are shown in table 4.2. Angiogenin was lower in White compared to South Asian and to Black groups. Compared to healthy controls, angiopoietin-2 was higher in the risk factor and the cardiovascular disease group. In the 56 healthy controls, only angiogenin varied between the ethnic groups: 6.35 (3.2-8.6) ng/mL in 18 South Asians, 5.3 (3.7-7.5) ng/mL in 18 Blacks, and 3.3 (1.7 – 6.0) in the 20 White ($p=0.043$ overall, $p<0.05$ South Asian vs. White). However, this finding was no longer apparent in subjects with at least one cardiovascular risk factor or subjects with cardiovascular disease. In fact, in subjects with one or more cardiovascular risk factors, none of the research indices were associated with ethnic/racial group.

Investigating possible associations between the research indices on univariate analysis, sTie2 correlated inversely with angiogenin ($r = -0.44$, $p=0.001$, Figure 4.1). No other univariate

associations were found between the research indices. sTie2 was lower in 16 smokers (87 (61-130) ng/mL) compared to 48 non-smokers (136 (100-166) ng/mL, p=0.007). sTie2 appeared to be higher in the women (124 (83-170) ng/mL) compared to the men (86 (75-133) ng/mL (p=0.029) although this was not significant and the possible effect of sex on sTie2 seen in healthy volunteers also became non-significant. There were no correlations where the coefficient exceeded 0.4.

In the 108 cases with stable cardiovascular disease, there was no effect of ethnicity, or of the number of symptomatic cardiovascular territories on the research indices, and no effect of smoking or sex on levels of sTie2. There were no correlations where the coefficient exceeded 0.4.

Table 4.2: Univariate Comparisons of Angiogenic Markers

4.2.1 Analysis by racial/ethnic group

4.2.2 Analysis by disease process

4.2.1

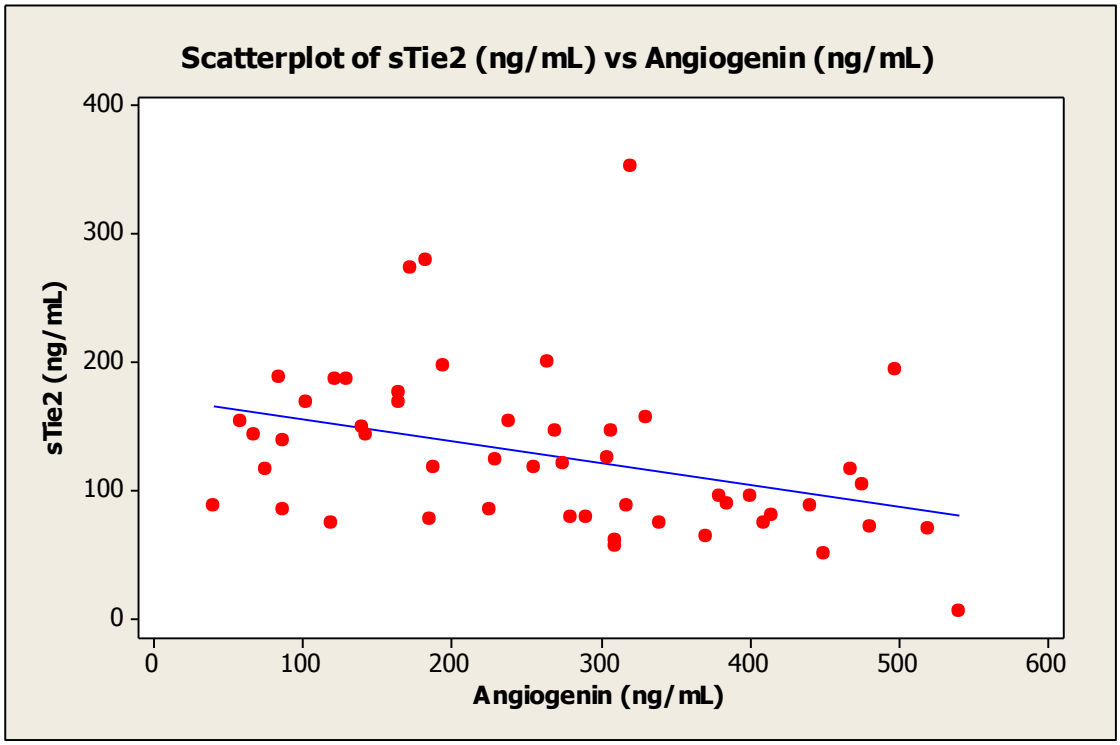
Angiogenic Marker	South Asian (n=82) [IQR]	Black (n=84) [IQR]	White European (n=77) [IQR]	p-value
Angiopoitein-1	16.8 [4.9-33.2]	12.4 [6.0-32.5]	9.4 [5.1-21.0]	0.246
Angiopoietin-2	1.3 [0.85-1.78]	1.2 [0.69-2.65]	1.35 [0.87-3.3]	0.301
sTie2	103 [79-146]	119 [80-168]	127 [83-166]	0.191
Angiogenin	240 [135-386]	261 [187-367]	169 [95-319]	0.01*

4.2.2

Angiogenic Marker	Healthy Controls (n=56) [IQR]	Risk Factors (n=64) [IQR]	Cardiovascular disease (n=108) [IQR]	p-value
Angiopoitein-1	14.8 [5.2-33.0]	12.4 [5.2-35.8]	12.3 [5.3-26.8]	0.950
Angiopoietin-2	0.88 [0.69-1.71]	1.32 [0.81-3.1]	1.52 [0.9-2.4]	0.006**
sTie2	117 [79-158]	126 [87-153]	114 [82-162]	0.851
Angiogenin	260 [137-372]	221 [114-314]	238 [135-371]	0.347

sTie2: soluble Tie-2 receptor; IQR: interquartile range; Units for all growth factors, ng/mL. Data analysed by the Kruskal-Wallis Test for non-parametric testing between 3 groups. *Lower in Whites compared to South Asians and to Blacks ($p<0.05$). Multivariate analysis showed this relationship to be present only in those free of risk factors or cardiovascular disease. **Higher in risk factors and cardiovascular disease ($p<0.05$). However, the effect of the disease spectrum on angiopoietin-2 disappeared in multivariate analysis.

Figure 4.1: Correlation between sTie2 and angiogenin in the healthy controls



r = -0.44, p=0.001

In multivariate analysis, general linear models identified age (p=0.001) to be the only independent factor associated with angiopoietin-1, whilst ethnic group (p=0.018) and SBP (p=0.01) were the only factors associated with angiogenin. In nominal regression analysis, SBP (p=0.014) and levels of angiogenin (p=0.01) both remained higher in the Black group compared to the White group.

4.1.6 Discussion

This study reports for the first time an analysis of circulating markers of angiogenesis in three different ethnic groups and the effect of risk factors and in stable atherosclerotic disease. It also investigated the inter-relationships between Ang-1, Ang-2, sTie2 and angiogenin.

This study found angiogenin levels to be significantly lower in healthy White than in both South Asian and Black groups. However, this effect disappeared in the presence of risk factors, and in stable cardiovascular disease, indicating some effect of the latter two processes. Others have reported raised angiogenin in acute coronary syndromes [Burgmann et al. 1996; Tello-Montoliu et al. 2006; Idriss et al. 2010], but not in stable coronary artery disease [Idriss et al. 2010] in predominantly White populations. This study now extends this by reporting no change in angiogenin in South Asians and Blacks with stable cardiovascular disease. Angiogenin has been thought to be an indicator of endothelial damage related to progression of vascular disease, it binds actin on the surface of endothelial cells, and its plasma levels are dependent on the presence of an angiogenic stimulus [Tello-Montoliu et al. 2006]. Its levels may therefore be expected to be associated with related molecules, and the correlation with sTie2 as found in this study may reflects this.

Several studies have suggested that angiogenin, and other angiogenic factors, could promote atherosclerosis and potentially de-stabilise plaques by promoting intra-lesional angiogenesis [Kolodgie et al. 2003; Khurana et al. 2005]. If so, this study's data suggest that plasma levels do not reflect this process. The higher levels in healthy asymptomatic middle aged (mean

age 56 years) South Asians and Blacks may in part explain the apparently higher cardiovascular disease burden in these ethnic groups when compared to the general population [Allison et al. 2006; Gill et al. 2007]. It is also possible that the higher angiogenin levels could in part be attributable to higher blood pressure and/or the higher prevalence of diabetes amongst the South Asians and Blacks with respect to the White groups.

The relationship between angiogenin and race/ethnicity present in health was only just lost in the presence of risk factors ($p=0.087$), but markedly so in stable cardiovascular disease ($p=0.595$), imply an effect to the latter two aspects on the growth factor. Such an effect may be effect of pathophysiology and/or drug therapy on the endothelium.

sTie2 was higher in the middle aged healthy women than in health men, precisely the reverse of a much larger community-based study, although the latter subjects were younger (and the women more likely to be pre-menopausal [mean age 40 years]) and had more disease [Lieb et al. 2010], although in this study this effect disappeared in risk factors and in frank disease. Some of this may be due to the effect of smoking. Notably, this finding parallels that of reduced plasma levels of the receptor for VEGF, feline McDonough Sarcoma-like tyrosine receptor-1 (Flt-1) in smokers [Belgore et al. 2000]. Curiously, this may have a real cardiovascular effect as smoking, and so lower Flt-1, protects against pre-eclampsia [Jeyabalan et al. 2008]. Failure to find altered sTie2 in stable cardiovascular disease contrasts with others who reported raised levels in acute coronary syndromes [Wang et al. 2005], suggesting that alterations in angiogenic markers are the result of the acute changes. Cleavage of the extra-cellular domain of Tie2 on endothelial cells occurs in response to inflammation and results in circulating sTie2, a process which is reduced by hypoxia [Van

der Heijen et al. 2010]. In acute inflammatory states, sTie2 in the plasma is bound by Ang-1 preferentially over Ang-2 due to its higher affinity for the receptor, leading to greater binding of Ang-2 to endothelial Tie2 and increasing endothelial permeability [Van der Heijen et al. 2010; Jones et al. 2001]. In hypoxic states, the relatively lower endothelial release of sTie2, leads to less Ang-1 binding within the plasma and greater endothelial binding, which accelerates vessel maturation [Post et al. 2008]. This preferential binding of Ang-1 over Ang-2 may explain the rise in plasma Ang-2 found in the presence of cardiovascular risk factors and cardiovascular disease seen in this study. However, whether or not these effects, prominent in cell biology, have any role in the pathophysiology of cardiovascular disease cannot be inferred from this study.

The only significant correlation was inversely between sTie2 and angiogenin. However, there seems to be no clear physiological or pathological relationship between the two. Further research into the possible relationship between these two angiogenic markers is required before firm conclusions can be made as to whether they have any physiological effect on each other.

Limitations

This study is limited by its cross-sectional design, but it still represents the first study to investigate ethnic differences in circulating markers of angiogenesis and their association with cardiovascular risk factors and frank, but stable, cardiovascular disease. Although this study has adjusted for various know risk factors, as well as age and gender, in the multivariate analysis, clearly residual confounding may still be present.

4.1.7 Conclusion

Ethnic differences are evident in circulating markers of angiogenesis, which may reflect susceptibility of particular groups to a greater predilection to cardiovascular disease. However, once cardiovascular disease or its risk factors are present, the value of these plasma markers diminishes.

CHAPTER 5

SUMMARY AND CONCLUSIONS

5.1 Summary of Results

In this thesis it has been demonstrated that ethnic differences exist in the prevalence of traditional cardiovascular risk factors, in pre-clinical atherosclerosis (CCIMT) and in peripheral arterial disease (PAD). It has also been shown that ethnic differences are apparent in markers of haemostasis and thrombosis and that different pathophysiological processes predominate in different ethnic groups.

Chapter 3.1 attempted to validate the Edinburgh Claudication Questionnaire in languages of the Indian sub-continent and also amongst 1st generation UK Black Caribbean migrants. It found the sensitivity and specificity and overall diagnostic accuracy of the ECQ was lower in Punjabi and Urdu translations and amongst Black Caribbeans than in the Edinburgh Artery Study but reflected the findings of other studies. It is unclear from this study whether the apparent lower sensitivities and specificities found are due to linguistic and cultural differences in describing the location and duration of pain or due to insufficient physical activity to bring on symptoms of intermittent claudication in the ethnic minority groups studied. It is also possible that the findings are the result of measuring ABPI, a more objective measure of claudication than was used in the Edinburgh Artery Study.

Chapter 3.2 investigated the prevalence of PAD in South Asians and Blacks. When comparing the two groups as a whole it found no significant differences in PAD prevalence. However, among female subjects, South Asians had a statistically significantly higher prevalence of this disease than Blacks. While both ethnic groups had equivalent levels of hypertension and diabetes, South Asian women had significantly lower levels of smoking. In previous research in predominantly White European populations it has been thought that abnormalities in the components of the lipid profile do not appear to carry the same importance as smoking and diabetes in the development of PAD [Faxon et al. 2004]. The most frequent dyslipidaemia associated with PAD is elevated triglycerides and low HDL-C [Smith et al. 2004]. In this study South Asian women had significantly higher triglycerides and lower HDL-C than their black counterparts. It might be possible that in South Asian women where the prevalence of smoking is low [Bhopal et al. 1999], dyslipidaemias are more important in the development of PAD. However causal associations cannot be made as a result of this cross-sectional study.

It's also possible that the South Asian women have less access to healthcare due to language barriers and high levels of illiteracy, even in their first language. The communication difficulties could mean they are less likely to be targeted by primary prevention strategies set out to reduce their cardiovascular risk. It's also possible that their increased prevalence of PAD is due to having more sedentary lifestyles than other ethnic groups [Hayes et al. 2006, Fischbacher et al. 2004].

Chapter 3.2 found the prevalence of diabetes and hypertension in both South Asian and Black participants was high overall and higher than in previous UK population based studies

[Chaturvedi et al. 1994; Diabetes in the UK 2004], with no significant differences between the two groups. However South Asians overall had a less favourable glycosylated haemoglobin level, which has been shown to be related to an elevated risk of incident PAD [UKPDS 1998].

With regards to the hypothesis that ethnic differences will exist in the associations between traditional cardiovascular risk factors and PAD, this thesis showed that male sex, SBP, diabetes and smoking were all independent predictors for PAD in South Asians after adjusting for traditional cardiovascular risk factors. In Blacks, only age was an independent predictor of this disease. This could suggest that cardiovascular risk factors are more closely related to each other in their effect on the pathophysiology of PAD in Blacks; although causal associations cannot be inferred from this cross-sectional study.

Chapter 3.3 investigated the hypotheses that ethnic differences will exist in mean and maximum CCIMT, a marker of pre-clinical atherosclerosis, and its relationship to cardiovascular risk factors and PAD. This chapter found that on univariate analysis Blacks, as a whole group, had higher mean CCIMT. This was also apparent in male and female subjects when analysed separately. On multivariate analysis, adjusting for traditional cardiovascular risk factors, Black ethnicity was a significant independent predictor of both mean and maximum CCIMT. This supports the possibility of a genetic/ ethnic predisposition to vascular disease in different vascular territories and supports previous research [D'Agostino et al. 1996; Kuller et al. 1998].

Chapter 3.3 also found ethnic differences in associations between CCIMT and traditional cardiovascular risk factors. In South Asians, the presence of hypertension was associated with greater mean and maximum CCIMT on univariate analysis as were SBP and PP. SBP remained an independent predictor of both mean and maximum CCIMT after adjusting for traditional cardiovascular risk factors. This finding was not found in the Black subjects in spite of equivalent levels of hypertension prevalence in both ethnic groups. Diabetes had univariate associations with mean and maximum CCIMT in both ethnic groups, as did HbA1c, but no longer remained an independent predictor of CCIMT on multivariate analysis. Maybe this is because subjects with diabetes are more likely to have co-existent risk factors, such as higher systolic blood pressure or are more likely to be older, which are more important risk factors than diabetes as predictors of raised CCIMT.

Interestingly no associations were found between smoking and CCIMT in Black subjects in this study even though Blacks had a higher prevalence of ever and current smoking than South Asians. Amongst the South Asians smoking had univariate associations with both mean and maximum CCIMT but on multivariate analysis was only an independent predictor of mean CCIMT. This finding could reflect the role smoking has on initiating and accelerating atherogenesis by altering components of Virchow's triad.

Finally, chapter 3.3 found univariate associations between PAD and mean and maximum CCIMT in both South Asian and Black subjects. Even after adjusting for traditional cardiovascular risk factors, ethnicity and age, PAD remained an independent predictor of both raised mean and maximum CCIMT on multivariate analysis, reflecting the greater cardiovascular burden in subjects with this disease.

Chapter 3.4 investigated the hypothesis that ethnic differences would be apparent in associations between markers of inflammation (CRP, IL-6) and haemostasis (sP-sel, VWF and fibrin D-dimer) and CCIMT and PAD. This chapter found differences in biomarker profile between South Asians and Blacks and reported different associations between biomarkers, CCIMT and ABPI. Notably, it found Blacks to have greater D-dimer levels than South Asians overall and in women. This study's findings support a previous study of raised D-dimer when compared to other ethnic groups in African Americans [Lutsey et al. 2006]. This finding suggests Black ethnicity may be associated with a greater predisposition to a hypercoagulable and prothrombotic state than South Asians.

Chapter 3.4 found South Asians had higher sP-sel and platelets than Blacks overall and when analysed by sex. It found Black women had higher VWF than South Asian women, supporting previous research [Kim et al. 2010]. In the South Asian subjects, VWF was associated with both mean and maximum CCIMT on multivariate analysis, after adjusting for traditional cardiovascular risk factors. This may reflect greater endothelial dysfunction/ damage and possibly a higher atherosclerotic load but not overt disease in South Asians.

Rather surprisingly no associations were reported between markers of inflammation and CCIMT and PAD in this chapter as IL-6 and CRP have previously been associated with PAD development, progression and severity of disease [Ridker et al. 2001; Devaraj et al. 2003; Tzoulaki et al. 2005] in predominantly White European populations. This study's findings could be a result of low levels of blood sampling or may reflect different pathophysiological processes predominating in different ethnic groups.

Chapter 4.1 investigated the hypothesis that ethnic differences will exist in the plasma expression of markers of angiogenesis (Angiopoietin-1, Angiopoietin-2, soluble Tie-2 receptor and Angiogenin) and their association with traditional cardiovascular risk factors and cardiovascular disease. This chapter also tested the hypothesis that the plasma levels of these biomarkers will be higher in subjects with traditional cardiovascular risk factors and higher still stable cardiovascular disease. This chapter found angiogenin levels were lower in healthy White subjects when compared to Blacks and South Asians but this finding disappeared in the presence of cardiovascular risk factors and stable cardiovascular disease, indicating some effect of the latter two processes on this biomarker. While it is possible that the increased angiogenin in Black and South Asian subjects could be partly attributed to the higher prevalence of hypertension and/ or diabetes, the fact that the apparent ethnic difference disappears in the presence of these conditions suggests these conditions are not the only reason for this difference.

Angiogenin is thought to be an indicator of endothelial damage related to the progression of vascular disease. Its levels may therefore be expected to be associated with related angiogenic molecules. This chapters finding of a negative correlation between angiogenin and sTie-2 may reflect this. In hypoxic states there is a reduction in the release of sTie-2 leading to less Ang-1 binding in the plasma and greater endothelial binding. As vascular disease progresses there is an increase in relative tissue hypoxia and an increase in plasma angiogenin. Chapter 4.1 failed to find any other ethnic differences in the plasma levels of the angiogenic markers investigated in the presence of traditional cardiovascular risk factors and stable cardiovascular disease.

5.2 Recommendations for Future Study

This thesis did not adjust for multiple significance testing in either the sub-study to E-ECHOES or the hospital based study. Reasons for this are that both studies were cross-sectional in their design, had small numbers of subjects in each cohort and the aims were to investigate possible associations between research parameters in areas where there is currently very little evidence due to limited research. Michels and Robins have previously stated that, "Cross-sectional studies should be used to find undiscovered associations so that further hypotheses can be generated for further investigation" [Michels & Robins 1996]. On speaking about epidemiological studies, Bland and Altman have previously suggested that the Bonferroni correction is highly conservative and may miss real differences [Bland & Altman 1995]. Taking these thoughts into account and realising that little is known about PAD in South Asians and Blacks and its associations with traditional cardiovascular risk factors and markers of haemostasis, inflammation and angiogenesis it was thought adjusting the level of significance could potentially miss real associations and limit further investigation into associations found in this thesis. Hypotheses generated as a result of findings from each chapter for future investigation will be discussed in turn below.

The findings of low sensitivity and specificity of the ECQ for diagnosing IC in South Asians and Blacks found in Chapter 3.1 has thrown up several questions. Notably could the difference in sensitivity and specificity simply be due to methodological differences in IC diagnosis between the Edinburgh Artery study and this thesis? A hypothesis to be tested would be: There will be a difference in the sensitivity and specificity of the ECQ at diagnosing intermittent claudication between a doctor made diagnosis of PAD and using

ABPI. A straightforward study could be designed using vascular surgeons assessing subjects with leg pain for possible IC using the ECQ, blinded to the ABPI of the study subjects. The sensitivity and specificity of the ECQ could then be calculated to see if differences in methodology result in differences in the ECQ's sensitivity and specificity.

The next question that has arisen is whether the difference in sensitivity and specificity is due to linguistic differences in describing pain. To test this difference amongst South Asian subjects, one could investigate bilingual subjects who could complete the ECQ in English and in their chosen South Asian language separated by a time interval, perhaps a week, randomised to whether they completed the ECQ in English or in a South Asian language first. Comparing the responses the subjects gave in the different language ECQ might allow assessment of any linguistic differences in completing the questionnaire.

It is also possible subjects completing the ECQ did not exercise sufficiently to experience symptoms of claudication in this thesis. Treadmill testing of subjects over a specified distance or time, with ABPI measurement before and after the session in conjunction with subjects completing the ECQ in their chosen language could investigate the apparent findings of low sensitivity and specificity found in this study. Finally, it's possible this thesis' findings are due to low statistical power from low recruitment of subjects with leg pain on exertion. Any future studies aiming to validate the ECQ may be better focused to include subjects referred to the vascular surgical department with leg pain rather than opportunistically asking everyone involved in a community based screening survey, such as the E-ECHOES study as focussed recruitment might yield a greater number of subjects with IC.

The original purpose of this thesis was to be a pilot study investigating PAD and its associations with traditional cardiovascular risk factors and CCIMT, a marker of pre-clinical atherosclerosis in the two largest minority ethnic groups in the UK as it had not been investigated before. A p-value <0.05 was deemed significant for the purposes of this thesis. It would be possible to investigate all possible associations found in this thesis with greater numbers of participants and a greater level of blood sampling to improve the power of the results and make more firm conclusions based on associations with PAD.

This thesis has reported differences in PAD prevalence between South Asian and Black women, while no difference was found when comparing the two ethnic groups as a whole or in men. It also found ethnic differences in CCIMT, with significantly higher CCIMT in Blacks. Based on power calculations made following the results of this study's estimate of PAD prevalence in South Asians and Blacks, a large prospective epidemiological study investigating the prevalence of PAD in these ethnic groups could be performed. Subjects would be included only if they consent for blood sampling. At baseline they would have all demographic details, lifestyle questionnaire, anthropometric measurements and blood sampling undertaken. Subjects could then be followed up at 5 yearly intervals and reassessed with further blood sampling and measurement of markers of inflammation, haemostasis and thrombosis. Over time, differences in these markers and their associations with the development of PAD could be assessed and further hypotheses generated from the research could be investigated further. This thesis found ethnic differences in markers of haemostasis and thrombosis. A prospective follow up study would determine with more accuracy if such differences are apparent and if so, give more insight into how these differences play a role

the development and progression of PAD South Asians and Blacks over time. A longitudinal study might also show that risk factor modification has an impact on these novel markers. Likewise, structured exercise programmes have previously shown improvements in ABPI and symptomatology. Assessing the effect of structured exercise programmes on the plasma levels of novel markers and their associations with ABPI and symptomatology may shed further light on the pathophysiology of PAD.

Another finding of this thesis which needs to be investigated further is the lack of an association between inflammatory markers and PAD in South Asians and Blacks, which goes against previous research [Ridker et al. 2001; Devararj et al. 2003; Tzoulaki et al. 2005]. Are the results of this thesis due to low levels of blood sampling or is inflammation a greater determinant of the development and progression of PAD in White populations? Could it be that there are ethnic differences in the pathophysiological processes involved the development and progression of PAD and that elevated markers of thrombosis are more important in Black subjects than in White populations? Only future, greater powered research might answer this question.

A longitudinal study could also look at whether maximum or mean CCIMT is more important in the progression of atherosclerosis and its symptomatic complications. If one is found to have greater prognostic significance over the other, it may lead to the incorporation of this measurement as a screening tool or indeed either mean or maximum CCIMT measurement may be used to monitor the effect of risk factor modification on this parameter over time.

Other future developments investigating possible ethnic differences in atherosclerotic vascular diseases could be focussed on a radiological diagnosis of disease. Rather than using a surrogate marker for PAD, such as ABPI, magnetic resonance angiography (MRA) or CT angiography (CTA) could be used to accurately measure the degree and distribution of atherosclerotic disease throughout the body in subjects from different ethnic groups and its progression over time. MRA although more costly, would not subject study participants to radiation or intravenous contrast that CT angiography would. Subjects could be screened for lower limb disease, abdominal aortic aneurysm, coronary artery disease and cerebrovascular disease. MRA or CTA could also be used to see whether there are differences in the 'normal' range of vessel calibre between ethnic groups, as this could be a factor in the predilection of atherosclerosis to different vascular territories in different ethnic groups. Indeed it may also be possible to investigate if there are ethnic differences in the burden of vascular disease in different vascular territories.

5.3 Conclusion

Peripheral arterial disease is an important healthcare problem accounting for significant morbidity and mortality. Previous PAD research has been performed in predominantly White European populations with relatively little research performed in non-White populations. While ethnic differences are apparent in cerebrovascular and cardiovascular disease, the answer as to whether ethnic differences exist in the prevalence of PAD is unknown. In an attempt to address the current dearth of literature on the subject, this thesis set out to investigate possible differences in the epidemiology and pathophysiology of PAD in the 2 largest minority ethnic groups living in the UK. The results reported in this thesis suggest differences in the prevalence of traditional cardiovascular risk factors and their associations with PAD and mean and maximum CCIMT.

This thesis also suggests there may be ethnic differences in the prevalence of PAD, especially amongst women, which cannot be explained by existing risk factor profile alone. Furthermore, this thesis also demonstrated for the first time ethnic differences in markers of haemostasis, thrombosis and angiogenesis and their relationship with PAD and CCIMT. Interestingly it found no associations between markers of inflammation and PAD, which goes against previous reports in predominantly White European populations.

In an attempt to assess the validity and applicability of using the ECQ in non-White populations (A healthcare questionnaire designed to diagnose IC in predominantly White European populations) this thesis investigated the ECQ in South Asian and Black subjects. This thesis reported differences in sensitivity and specificity to results found in the original

Edinburgh Artery Study. It is possible that methodological differences, linguistic and cultural differences in the description of pain or lack of sufficient exertion to bring about the onset of symptomatic PAD in the form of IC, could explain this thesis' findings.

In summary, this thesis has already furthered our understanding of possible ethnic differences in the epidemiology and pathophysiology of PAD in South Asians and Blacks living in the UK, in this relatively under-investigated field; however clearly further investigation is needed. This thesis' contribution to the existing medical literature is evidenced by the fact that every data chapter has been published in peer reviewed journals.

LIST OF PUBLICATIONS ARISING FROM THIS THESIS

Bennett, P.C., Lip, G.Y.H., Silverman, S.H., Blann, A.D., Balakrishnan, B., & Gill, P.S. (2012) Ethnic/Racial Differences in Circulating Markers of Angiogenesis and their Association with Cardiovascular Risk Factors and Cardiovascular Disease. *Int.J.Cardiol., In Press.*

Bennett, P.C., Lip, G.Y.H., Silverman, S.H., Blann, A.D., & Gill, P.S. (2011) Validation of the Edinburgh Claudication Questionnaire in 1st generation Black African-Caribbean and South Asian UK migrants: A sub-study to the Ethnic-Echocardiographic Heart of England Screening (E-ECHOES) Study *B.M.C.Med.Res.Methodol.*, **3**, 11:85.

Bennett, P.C., Gill, P.S., Silverman, S., Blann, A.D., Chackathayil, J., & Lip, G.Y. (2011) Haemostatic cardiovascular risk factors, common carotid intima medial thickness and peripheral arterial disease in South Asians and African-Caribbeans: a sub-study to the Ethnic-Echocardiographic Heart of England Screening (E-ECHOES) Study. *J.Thromb.Haemost.* **9**, 645-652.

Bennett, P.C., Gill, P.S., Silverman, S.H., Blann, A.D., & Lip, G.Y.H. (2011) Ethnic differences in Common Carotid Intima Media Thickness, and the relationship to cardiovascular risk factors and peripheral arterial disease: The Ethnic-Echocardiographic Heart of England Screening (E-ECHOES) Study. *Q.J.M.,* **104**, 245-254.

Bennett, P.C., Lip, G.Y.H., Silverman, S.H., Blann, A.D., & Gill, P.S. (2010) The contribution of Cardiovascular Risk Factors to Peripheral Arterial Disease in South Asians and Blacks, a sub-study to the Ethnic-Echocardiographic Heart of England Screening (E-ECHOES) Study *Q.J.M.,* **103**, 661-669.

Bennett, P.C., Lip, G.Y.H., Silverman, S.H., & Gill, P.S. (2010) Chapter 15: Peripheral Vascular Disease and Angiogenesis: Is there a link between angiogenesis and atherothrombosis? In Therapeutic Angiogenesis (Springer) Ed. Slevin, M.

Bennett, P.C., Silverman, S.H., Gill, P.S., & Lip, G.Y.H. (2009) Peripheral arterial disease and Virchow's Triad. *Thromb.Haemost.,* **101**, 1032-1040.

Bennett, P.C., Silverman, S.H., Gill, P.S., & Lip, G.Y.H. (2009) Peripheral arterial disease in ethnic minority groups. *Q.J.M.,* **102**, 3-16.

LIST OF PRESENTATIONS ARISING FROM THIS THESIS

Association of Surgeons in Training (ASIT) 2011 (Sheffield)
Bennett, P.C.**,** Gill, P.S., Blann, A.D., Silverman, S.H., & Lip, G.Y.H. Ethnic differences in circulating markers of angiogenesis and their association with cardiovascular risk factors and peripheral arterial disease – *Abstract published in International Journal of Surgery (IntJ.Surg.,* **9**,496-497).

European Atherosclerosis Society Congress 2010 (Hamburg)
Bennett, P.C., Silverman, S.H., Gill, P.S., & Lip, G.Y.H. Common Carotid intima media thickness is independently associated with peripheral arterial disease in UK 1st generation Asian and Black Migrants – *Abstract published in Atherosclerosis Supplement (Atherosclerosis.Suppl.,* **11**,18).

Vascular Society of Great Britain & Ireland AGM 2009 (Liverpool)
Bennett, P.C., Silverman, S.H., Gill, P.S., & Lip, G.Y.H. Hypertension and diabetes mellitus as cardiovascular risk factors for peripheral arterial disease in South Asian migrants: the Ethnic Echocardiographic Heart of England Screening Study (E-ECHOES)

APPENDIX I.

Consent form for Hospital Based Study

Comparison of plasma versus histological indices of angiogenic markers in atherosclerosis
(Comparison between the blood and blood vessel wall levels of special proteins in patients with hardening of the arteries)

CONSENT FORM

Name of Researcher: _____Patient Identifier_____

Please initial box

I confirm that I have read and understand the information sheet dated 01/05/08 (Version 2.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

I understand that relevant sections of my medical notes and data collected during this study may be looked at by individuals from Sandwell and West Birmingham Hospitals NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

I understand that my personal details will be held by the Department on computers that are Subject to the Data Protection Act, and that such details are confidential to the members of the research team. ☐

I understand that the blood and tissue samples I give will be kept until the end of the study year and then destroyed ☐

I agree to my GP being informed of my participation in the study ☐

A copy of the information sheet and signed consent form will be given to you to keep.

By signing hereunder I accept the contents of this consent form and agree to participate in this study voluntarily.

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

SECTION BELOW TO BE COMPLETED IF NECESSARY

_____	_____	_____	_____
Impartial Witness Name	Relationship to patient	Signature	Date

One copy for researcher (original), one for patient, one for patient notes.

APPENDIX II.

Participant Information Sheet

This information sheet is in two parts. Part 1 provides brief and clear information on the essential elements of the specific study: what the research is about, the condition or treatment under study, voluntary nature of involvement, what will happen during and after the trial, what treatment may be withheld, the participant's responsibilities, the potential risks, inconvenience or restrictions balanced against any possible benefits.

Part 2 contains additional information on factors such as confidentiality and data protection, communication with the GP, indemnity and compensation, etc., which should, of course, be read and understood before the participant decides whether they want to participate.

Part 1 of the information sheet

1. **Study title**

Comparison of plasma versus histological indices of angiogenic markers in atherosclerosis (Comparison between the blood and blood vessel wall levels of special proteins in patients with hardening of the arteries)

2. **Invitation paragraph**

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

3. What is the purpose of the study?

Peripheral artery disease (narrowing of the blood vessels) is an important healthcare problem in developed nations and is the commonest cause of stroke, non-cardiac sudden death and amputation. Peripheral artery disease is important because it is an indicator of widespread atherosclerosis (hardening of the blood vessels) in other places in the body such as the brain and heart. Atherosclerosis is associated with angiogenesis (The formation of new blood vessels). Angiogenesis is controlled by a number of different special proteins in the blood and in the blood vessel wall. We aim to investigate the levels of some of these special proteins contributing to angiogenesis both in the blood and in the blood vessel wall (From the specimen we collect) to see if their levels are related to each other.

4. Why have I been chosen?

You have been chosen because you are due to have surgery for peripheral artery disease and there are likely to be left-over blood vessel specimens that would normally have been thrown away.

5. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

6. What will happen to me if I take part?

If you take part, on the day of your operation before your surgery we will take a small sample of your blood. Any left over blood vessels, that would normally be removed during your operation and thrown away, will be collected and stored as part of our study. We will perform tests on the blood and blood vessel wall at a later date. We will not take any blood vessel specimens from you that have not already been removed as part of your surgery.

If you are having carotid end-arterectomy (surgery on the neck blood vessels) you will be invited to City Hospital to have a special scan of your heart. This scan involves putting jelly on your chest allowing us to see the heart. There is a very small chance of detecting a problem if you have no previous history of heart disease. If we detect a problem we will organise any follow up that you might require with the heart specialists at Sandwell and West Birmingham hospitals NHS Trust.

7. What do I have to do?

You will be asked to donate a small sample of your blood before your operation. You will have the surgery that you have agreed to previously. The left over blood vessel will be collected during your operation.

If you are booked for a carotid end-arterectomy you will be invited to attend Birmingham City Hospital at another date before your surgery to have a special scan of your heart, the results of which will be sent to your GP.

All samples we obtain from you will be kept until the end of the study year and then destroyed.

9. Are there any side effects/ risks or disadvantages of taking part?

Apart from the risk of a small bruise you may get from having blood taken there will be no added risks from being in this study. The usual risks and complications of your operation will apply to you.

11. What are the possible benefits of taking part?

There will be no direct benefit to you for taking part but the information we get from this study will help to improve our understanding of the causes of atherosclerosis, the main cause of PAD. This may benefit people in the future.

12. What will happen to the results of the research study?

The results will be published in medical journals and you will not be identified in any report or publication.

13. What happens when the research study stops?

Your routine care will continue regardless of the duration of this study.

Part 2 of the information Sheet

14. What if new information becomes available?

Sometimes during the course of a research project, new information becomes available. If new potential markers for angiogenesis (New blood vessel formation) are identified we may test the blood and blood vessel specimens we receive from you for these. This will not alter your treatment in any way.

15. What if something goes wrong?

Taking blood is a routine procedure with very little risk. You will have had blood taken before and therefore will be aware that very little can go wrong. With regards to your blood vessel specimen, we will only be collecting a sample from you that would otherwise be thrown away during your procedure.

If you are invited to have a scan, this involves having gel put on your chest in order for the scanner to see your heart. This will not cause you any harm.

If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

16. Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital/surgery will have your name and address removed so that you cannot be recognized from it.

All data will be stored on a password protected database in the University Department of Medicine at Birmingham City Hospital. Data will only be accessible to members of the study team.

17. Who has reviewed the study?

Birmingham East North and Solihull Ethics Committee

18. Will my General Practitioner be informed?

Your General practitioner will be informed of your decision to participate in the study. If you are having a heart scan your GP will be informed of the result.

19. Contact for Further Information

If you wish to discuss this study further then you may contact the following:

Philip Bennett Research Fellow

Stanley Silverman Consultant Vascular Surgeon [redacted]

If you want independent advice about whether or not to participate in this study you may contact the following through the hospital switchboard (0121 5543801):

The Patient Advice and Liaison Service (PALS)
The Research and Development Office at Sandwell or City Hospitals
Dr Siddique Cardiology Research Fellow

You are under no obligation whatsoever to participate, and your decision would in no way influence your care and medical treatment in hospital.

APPENDIX III.

Letter to GP

Patient Identifier Label:

Dear Doctor,

The above patient has consented to participate in a study investigating angiogenesis in peripheral arterial disease at Sandwell and West Birmingham Hospitals NHS Trust. The study will involve taking a blood sample from the patient and discarded arterial tissue obtained during their procedure. Patients having carotid end-arterectomy will be invited to attend City Hospital on another occasion to have echocardiography. The patient will not be followed up as part of this study but will be followed up routinely by the vascular team. The study will not alter the management of this patient but may help in the future management of patients with peripheral arterial disease.

If you have any concerns do not hesitate to contact me on the above number.

Yours sincerely,

Philip Bennett MBChB MRCS
Vascular Surgery Research Fellow

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