Comparison of carotid plaque characteristics, arterial remodelling changes, left ventricular geometry and inflammatory markers in patients with chest pain and unobstructed coronary arteries, chronic stable angina or acute coronary syndromes

A thesis submitted to the University of Manchester for the Degree of Doctor of Medicine in the Faculty of Medical and Human Sciences

2013

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Abstract

The following thesis is submitted to the University of Manchester for the degree of Doctor of Medicine by Dr Satheesh Balakrishnan Nair on 15.08.2013.

Introduction: Atherosclerosis remains asymptomatic until it progresses to cause flow-limiting disease. Identifying patients at high risk in the early stages of the atherosclerotic process may allow modification of cardiovascular risk by effective preventive strategies. Various non-invasive tests have been studied and have shown promising results in predicting future adverse cardiovascular events. The objective of this study was to establish the carotid ultrasonographic markers that best correlate with angiographic coronary artery disease (CAD) and the relationship between left ventricular geometry, carotid atherosclerosis, biomarkers and CAD in patients with unobstructed coronary arteries, chronic stable angina (CSA) and acute coronary syndromes (ACS).

Methods: Carotid ultrasound examination, echocardiography and serum biomarker estimation were performed in consecutive patients who underwent coronary angiography for evaluation of stable or acute chest pain.

Results: A total of 146 subjects were recruited into the study with a mean age of 56.9 ± 10.6 (range 29 to 85) years; 120 were men (82%) and 26 (18%) women. Twenty-one percent of the study population had unobstructed coronaries, 42% had stable CAD and 37% had presented with ACS. There was no significant difference in the carotid intima-media thickness (CIMT) measurements between the three groups. CIMT correlated with abnormal left ventricular geometry but not with the presence or severity of CAD. The presence of carotid plaque and plaque score correlated with obstructive CAD, but was not significantly different between stable CAD and ACS patients. There was a trend towards more echogenic plaque in the stable CAD group. The composite score of IMT and plaque was positively correlated with the presence and severity of CAD. The averaged myocardial peak systolic and early diastolic velocities were significantly lower in those with obstructive CAD. CRP and osteopontin levels were higher in the ACS patients.

Conclusions: Carotid plaque and not CIMT was associated with angiographic coronary artery disease. Averaged systolic and early diastolic myocardial velocities by tissue doppler imaging correlated with obstructive CAD. Novel serum biomarkers are promising and further studies are needed.
Declaration

I declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

Satheesh Balakrishnan Nair

School of Medicine

Faculty of Medical and Human Sciences
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Dedication

I dedicate this thesis to my parents.

It would have been impossible for me to have reached this level without their sacrifices and constant encouragement.

Thank you for giving me the luxury of education.
Acknowledgements

I would like to thank Dr Rajdeep Khattar for giving me the opportunity to be part of this wonderful project. I am greatly indebted to him for his excellent guidance and supportive supervision during the period of research.

I also would like to thank Prof Rayaz Malik for his constant support, encouragement and guidance.

My sincere thanks to Prof Bernard Clarke for his kind support and guidance.

I would like to express my gratitude to Dr Stephen Roberts for his invaluable advice and supervision of statistical analysis.

I would like to thank Dr Valentine Charlton-Menys, Dr Handrean Soran and their team for the guidance and analysis of serum biomarkers.

My sincere thanks to the Research Nurses Heather, Karen, Kirsty and Wendy Specialist Nurses Gillian, Samantha and Justine for their kind help in facilitating patient recruitment.

I would also like to express my thanks to colleagues Sanoj, Matthew, Reza and Uazman for their help on various aspects of my research.

Last, but by no means the least, this work would not have been possible without the unconditional love and support from my wife, Surya and my beloved daughter, Neha.
Statement about the author

I graduated from DR MGR Medical University, India with a MBBS degree in 1996 and did further post graduate training in Medicine with the same University and obtained the degree, MD General Medicine in 1999. In the United Kingdom, I worked as Senior House Officer in Royal Infirmary, Edinburgh and The Cardiothoracic Centre, Liverpool. I passed my MRCP (UK) in 2004 and worked as Clinical Fellow in Cardiology at Manchester Heart Centre. I registered for the MD programme with the University of Manchester in September 2008. I obtained a training position in Cardiology in the Mersey Deanery in 2010 and I am currently pursuing my Specialist training in Cardiology with a view to completing the training in 2015.

In this study, I was responsible for designing the study protocol, obtaining ethical approval, and recruitment of study participants. After the initial clinical assessment, I personally performed and later analysed the echocardiographic and carotid ultrasound examinations of each patient. I collected the blood samples from the patients, centrifuged them and stored them in deep freezers for later analysis. I collected and managed the patient data and did the statistical analysis, which was supervised by the study statistician.
### List of common abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Expansion</th>
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<tbody>
<tr>
<td>ACS</td>
<td>Acute coronary syndrome</td>
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<tr>
<td>AHA</td>
<td>American Heart Association</td>
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<tr>
<td>Apo A1</td>
<td>Apolipoprotein A1</td>
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<tr>
<td>ARIC</td>
<td>Atherosclerosis Risk in Communities</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
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<tr>
<td>CB</td>
<td>Carotid bulb</td>
</tr>
<tr>
<td>CCA</td>
<td>Common carotid artery</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>CIMT</td>
<td>Carotid intima-media thickness</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross sectional area</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>ECA</td>
<td>External carotid artery</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>GCS</td>
<td>Global circumferential strain</td>
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<tr>
<td>HDL-C</td>
<td>High density lipoprotein-cholesterol</td>
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<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>High sensitivity C-reactive protein</td>
</tr>
<tr>
<td>ICA</td>
<td>Internal carotid artery</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischaemic heart disease</td>
</tr>
<tr>
<td>IMT</td>
<td>Intima media thickness</td>
</tr>
<tr>
<td>IMT-max</td>
<td>Intima media thickness including plaque</td>
</tr>
<tr>
<td>IQR</td>
<td>Inter quartile range</td>
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<tr>
<td>IVSD</td>
<td>Inter ventricular septal thickness in diastole</td>
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<tr>
<td>LA</td>
<td>Left atrium</td>
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<tr>
<td>LAV</td>
<td>Left atrial volume</td>
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<td>LAVI</td>
<td>Left atrial volume index</td>
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<tr>
<td>LDL-C</td>
<td>Low density lipoprotein-cholesterol</td>
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<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
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<td>LVH</td>
<td>Left ventricular hypertrophy</td>
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<td>LVIDD</td>
<td>Left ventricular internal dimension in diastole</td>
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<td>LVMI</td>
<td>Left ventricular mass index</td>
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<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>No-CAD</td>
<td>No obstructive coronary artery disease</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>Non-ST segment elevation myocardial infarction</td>
</tr>
<tr>
<td>OPN</td>
<td>Osteopontin</td>
</tr>
<tr>
<td>PON 1</td>
<td>Paraoxonase 1</td>
</tr>
<tr>
<td>PWTD</td>
<td>Posterior wall thickness in diastole</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristics</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST-elevation myocardial infarction</td>
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CHAPTER 1
Chapter 1. Introduction

1.1 An overview of coronary artery disease

1.1.1 Epidemiology of coronary artery disease

Atherosclerotic cardiovascular disease (CVD) is a diffuse disease involving the arterial circulations of the heart, brain, kidneys and limbs. This process begins in childhood and progresses over decades. Coronary artery disease (CAD) contributes to approximately one third to one half of total CVD. In the Framingham study, the lifetime risk for CAD in adults at age 40 was 49% for men and 32% for women (1). Ischaemic heart disease is the leading cause of death worldwide and the rate of CAD is on the rise due to a range of contributing factors including an ageing population, increased prevalence of obesity, type 2 diabetes and the metabolic syndrome. The World Health Organization has estimated that by 2020, the global number of deaths from CAD will have increased from 7.2 million in 2002 to 11.1 million (2).

Atherosclerosis remains asymptomatic until it progresses to cause flow limiting disease (plaque stenosis of 70 -80%) or when a thrombus forms on an existing plaque as a result of rupture or erosion (3). However, a substantial proportion of patients progress abruptly from inapparent disease to a myocardial infarction or possible death. This is often due to rupture of plaques with less than 50% stenosis. This has been demonstrated in studies of patients presenting with an acute coronary syndrome who had undergone coronary angiography recently, prior to the event, which had shown that the artery involved was only moderately diseased (4-6).

Identification of patients at high risk in the early phase is critical to allow for modification of cardiovascular risk by effective preventive strategies. Coronary
angiography is currently considered the gold standard for detecting obstructive coronary artery disease. However, its inability to detect early atherosclerotic changes, its invasive nature, as well as exposure to ionizing radiation, make it an unattractive primary screening tool in preventive cardiology. Various non-invasive tests have been studied and have shown promising results on their predictive value for future adverse cardiovascular events. Carotid intima-media thickness (CIMT), flow mediated dilatation of the brachial artery (FMD), augmentation index (AI), pulse wave velocity (PWV) are a few markers which have been studied extensively in primary prevention. Also, a number of biomarkers such as C-reactive protein (CRP) and fibrinogen have been found to be significantly related to subclinical atherosclerosis.

1.1. 2 Pathogenesis of Atherosclerosis

The arterial wall consists of three layers: the intima, the media, and the adventitia. The intima is the innermost layer and consists of a single layer of endothelial cells, which are in direct contact with blood flow. The media consists mainly of smooth muscle cells and constitutes most of the thickness of the arterial wall. The adventitia is the outermost layer and is mainly composed of collagen. The first phase in atherosclerosis histologically presents as a focal thickening of the intima with an increase in smooth muscle cells and extracellular matrix (7). This is followed by accumulation of intracellular or extracellular lipids or both which develops into a fatty streak. As these lesions expand, more smooth muscle cells migrate into the intima. The smooth muscle cells within the deep layer of the fatty streak are
susceptible to apoptosis, which is associated with further macrophage infiltration, and cytoplasmic remnants that can calcify, perhaps contributing to the transition of fatty streaks into atherosclerotic plaques (8). The fibrous plaque evolves from the fatty streak via accumulation of connective tissue with an increased number of smooth muscle cells, which are laden with lipids and often a deeper extracellular lipid pool. More advanced lesions become revascularized from both the luminal and medial aspects, and often contain a necrotic lipid rich core, which can eventually become calcified (9).

Atherosclerotic lesions are associated with structural changes in the vessel wall, referred to as arterial remodelling as shown in figure 1.1. In the early phase, there is arterial expansion at the sites of the coronary atherosclerotic lesion, which has been demonstrated, in animal models (10, 11). This is called positive remodelling, which is defined as a positive correlation between plaque and external elastic membrane (EEM) area due to a compensatory increase in local vessel size in response to increasing plaque burden (dilated atherosclerosis). There is no loss of lumen size at this stage. Glagov et al demonstrated this phenomenon in a necropsy study of 136 human coronary arteries where luminal area was not affected until the lesion reached 40% area stenosis (12). Negative remodelling refers to a smaller external elastic membrane area at the lesion site due to the local shrinkage of vessel size (obstructive atherosclerosis) (13, 14). Positive remodelling was initially described as a compensatory phenomenon, but studies with intra vascular ultrasound (IVUS) and coronary angioscopy have demonstrated that positive remodelling is more common in complex unstable plaques, in patients presenting with unstable angina; in contrast negative remodelling is
associated with smoother, stable plaques in patients with stable angina (15). The remodelling patterns are influenced by haemorheologic conditions (flow, wall stretch and sheer stress) and humoral factors (cytokines and vasoactive substances) (16, 17).

**Figure 1.1:** Vascular remodelling patterns across the different spectrum of CAD.

![Vascular remodelling pattern diagram](image)

EEM= external elastic membrane, CAD= coronary artery disease (Glagov et al (12)).

1.2 Carotid Ultrasound Scanning

1.2.1 Background

In recent years, ultrasound techniques have been developed extensively to detect the early phase of atherosclerosis. Carotid intima-media thickness
(CIMT) and carotid plaque assessment have emerged as valuable tools to
detect and monitor progression of atherosclerosis.

1.2.2 Carotid Intima Media Thickness

It is non-invasive, relatively simple to measure and the results are
reproducible with low inter and intra observer variability, allowing for
monitoring progression of the atherosclerotic process over time (18). Through
direct visualization of the arterial wall of a superficial artery like the carotid
artery, B-mode ultrasound can measure this thickening. The intima-media
thickness, defined as the thickness between the intimal-luminal and the
medial-adventitial interfaces, is measured as shown in figure 1.2. Ultrasound
imaging cannot discriminate between the intima and media layers because of
insufficient axial resolution. Therefore, an elevated CIMT could be the result of
a thickened intimal layer from atherosclerosis, an increased medial layer due
to vascular hypertrophy, or both. The validity of the B-mode method of
measurement of CIMT is well established by comparison with histology (19). It
utilizes the gray-scale appearance of the “double-line density” of the intima-
media complex and the adventitia of the artery (20). Its relationships with
coronary artery disease risk factors and future cardiovascular events allow for
it to serve as a surrogate for underlying coronary atherosclerosis (21-24).
Although it is currently primarily used as a research tool, it has the potential
for a clinical role in both primary and secondary prevention of CAD.
Figure 1.2: Ultrasound image of the carotid system showing carotid arterial layers.

The distance between the two arrows (black and white) is the intima-media thickness in the far wall of the common carotid artery.

1.2.3 Methodology of carotid intima-media thickness measurement

Although the general principles of carotid IMT measurement are common to all B-mode ultrasound studies, the methodologies used in these studies vary considerably with regard to image acquisition and analysis as shown in Table 1.1. Measurements of IMT can be taken from the common carotid artery (CCA), carotid bifurcation (bulb) and internal carotid artery (ICA). The schematic diagram of the carotid system is shown in figure 1.3. The common carotid artery (CCA) is the long tubular structure. The segment between the point where the CCA dilates and to the point where the flow divides is the carotid bulb. Then it bifurcates into the internal carotid artery (ICA) and the external carotid artery (ECA). Since the CCA is a tubular structure and is perpendicular to the ultrasound beam, measurement yield and reproducibility
of IMT in this region are greater than for IMT measurements in the bulb or ICA (25-27).

Both near and far walls can be visualized on B-mode scans, but studies comparing ultrasound measurements with histology suggest that far wall carotid IMT measurements are more representative of the true thickness of the arterial wall (19, 28, 29). Near wall IMT measurements, in comparison, are limited by their dependence on the axial resolution and gain settings of the equipment used and hence are less accurate and reproducible (21). The quantification of IMT values may be divided into two methodologies: measurement of the mean IMT and maximum IMT. The mean IMT is estimated as the mean of all the IMT measurements made over single or multiple segments of the carotid arteries, from the right and left sides and from the near and far walls of the arteries. The maximum IMT is the highest value of IMT measured over the carotid artery segments. As shown in Table 1.1, large-scale observational studies have used varying methodologies in single or multiple carotid artery segments (21-24, 30, 31).

Electrocardiographic gating is crucial for measuring IMT and lumen diameter since these parameters vary during the cardiac cycle. During systole, lumen diameter expands with a degree of thinning of IMT because of longitudinal stretching, and the opposite occurs during diastole (32, 33). Hence, standardised measurement techniques are especially important in clinical trials assessing the magnitude of change in IMT values over time. The American Society of Echocardiography recommends using end-diastolic measurements of carotid IMT.
In earlier studies, IMT measurements were performed by visually detecting the leading edges of the blood-intima and media-adventitia interfaces. However, recent studies have used a computer-based automated edge detection method. This involves determining ultrasound interfaces using pixel intensity and employs a multi-step gradient-based algorithm to accurately identify the intima-media complex. This technique distinguishes IMT values to within 0.01 mm and reduces operator dependency, thereby improving the reproducibility rates of measurements.

Several studies have verified the reproducibility of carotid IMT measurements. A review of 23 studies found intra-observer variability in the selected studies to be between a mean +/- SD difference of 0.02 ± 0.02 mm and 0.66 ± 1.13 mm, and an error percentage of 2.5% and 15.9%. The inter-observer variability was between a mean ± SD difference of 0.01 ± 0.04 mm and 0.65 ± 0.69 mm, with an error percentage of 5.9% and 13.7%. Variability was less when measurements were made in the CCA rather than the ICA and bulb, when mean rather than maximum IMT measurements were recorded, and when using an automated edge-tracking method as opposed to manual measurements (34).

1.2.4 Normal IMT Values

Normal values have been defined based on their distribution within a general healthy population and have been classified according to age and gender (22, 30). The definition of the upper limit of normal is arbitrary but is frequently set at the 75th percentile of CIMT distribution for the determination of increased relative CVD risk. Data from the Atherosclerosis Risk In Communities (ARIC)
study suggest that a value \( \geq 1 \) mm of IMT is associated with a significantly increased absolute risk of CAD (22). However, having a single threshold for an abnormal value will result in under detection of disease in the young and over detection in the older population and of course CIMT is a continuous variable, and the transition to focal plaque is arbitrary. Some investigators have suggested the normal range of CIMT to be 0.5 to 1.2 mm and have defined plaques as CIMT greater than 1.2 mm (35, 36). Another frequently used definition for plaque is a focal increase in CIMT greater than 1.5 times the surrounding CIMT (37). The Mannheim carotid IMT consensus defines plaque as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or demonstrates a thickness of >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface (38). Consensus statement from the American Society of Echocardiography 2008 suggests reporting CIMT values greater than or equal to 75\(^{th}\) percentile as indicative of increased CVD risk, 25\(^{th}\) to 75\(^{th}\) percentile as average or unchanged CVD risk and less than or equal to 25\(^{th}\) percentile to be at lower risk (39).

1.2.5 Prevalence and distribution of increased carotid intima-media thickness

As a consequence of the variable methodologies and different populations used in the measurement of IMT in previous studies, there is no clear cut-off point of IMT that defines arterial hypertrophy. In the Atherosclerosis Risk In Communities (ARIC) study (26), involving around 15,000 subjects aged 45-64
years, the mean IMT values from the distal common carotid artery, carotid bulb and internal carotid artery were estimated and this demonstrated similar age-related, arterial segment specific distribution patterns of carotid IMT. Individuals tended to have greater IMT in the carotid bulb than in the common carotid artery. Internal carotid artery measurements were more variable, with higher proportions of both smaller and larger IMT than in the common carotid artery. These differences between arterial segments reflect the physiological increase in wall thickness due to haemodynamic stresses in bifurcation and branching zones, as well as the comparatively greater frequency of atherosclerotic lesions in these locations.
Table 1.1: Overview of carotid IMT measurement methodologies in observational studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Arterial segment</th>
<th>IMT definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIHD (Kuopio Ischemic Heart Disease Risk Factor Study)</td>
<td>CCA</td>
<td>Max IMT near + far wall</td>
</tr>
<tr>
<td>Salonen et al 1993</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARIC (Atherosclerosis Risk in Communities study)</td>
<td>CCA, Bif, ICA, combined</td>
<td>Mean IMT far wall</td>
</tr>
<tr>
<td>Chambless et al 1997</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotterdam study</td>
<td>CCA</td>
<td>Mean IMT near + far wall</td>
</tr>
<tr>
<td>Bots et al 1997</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHS (Cardiovascular Health Study)</td>
<td>CCA, ICA, combined</td>
<td>Max IMT near + far wall</td>
</tr>
<tr>
<td>O’Leary et al 1999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kitamura et al 2004</td>
<td>CCA, ICA, combined</td>
<td>Max IMT near + far wall</td>
</tr>
<tr>
<td>CAPS (Carotid Atherosclerosis Progression Study)</td>
<td>CCA, Bif, ICA</td>
<td>Mean IMT far wall</td>
</tr>
<tr>
<td>Lorenz et al 2006</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CCA (common carotid artery); Bif (carotid bifurcation); ICA (internal carotid artery).

1.2.6 Determinants of Carotid IMT

1.2.6.1 Age and Sex

Age and sex have been found to be major determinant of arterial wall thickness in all clinical and epidemiological studies. In the ARIC study (26), at all ages, men have higher mean IMT values than women and IMT increases with age in men as well as in women at all arterial segments. The rate of progression of IMT in the common carotid artery is approximately 0.01 mm/year and this is similar in both men and women. However, the rates of progression are higher at the bifurcation and the internal carotid artery, ranging from 0.015 to 0.020 mm/year, reflecting the earlier and steeper
development of atherosclerotic lesions at these arterial locations. In the Cholesterol Lowering Atherosclerosis Study (CLAS) (40), the IMT increase was estimated to be 0.03-0.04 mm over 2 years and this is much lower than the 0.12 mm observed over 2 years, in the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) (41).

1.2.6.2 Blood Pressure
Numerous studies have examined the relationship between arterial wall thickness and either blood pressure or hypertension. All case-control studies have found that hypertensive subjects have significantly greater values of IMT compared to normotensive subjects (42-46). The mean difference in IMT between case and control subjects was in the range of 0.06-0.25 mm. Also, cross-sectional studies and studies on selected population have found a significant correlation with systolic blood pressure (43, 47, 48). However, the correlation with diastolic blood pressure has been found to be inconsistent. Some studies found a positive association with IMT (49, 50), whereas other studies found no association (43, 47, 51, 52).

1.2.6.3 Diabetes
Several cross sectional studies have shown that carotid IMT values are significantly greater in diabetic subjects when compared to non diabetic subjects. The Insulin Resistance and Atherosclerosis (IRAS) study (53) showed that diabetic patients without CAD had similar IMT values to non diabetic patients with CAD. This study also found that the progression of IMT was 25% greater in diabetic patients when compared with non diabetic
patients, even after correcting for known cardiovascular risk factors. A meta analysis of twenty one studies involving patients with type 2 diabetes by Brohall et al (54) found that diabetic subjects had 13% greater IMT values compared to non diabetic subjects. This was found in both men and women, as well as in Caucasians and other ethnicities. Carotid IMT values were also greater in subjects with impaired glucose tolerance (IGT), although to a lesser extent.

1.2.6.4 Smoking
Smoking has been found to be associated with increased carotid IMT in many studies (47, 49, 52, 55-57). The number of pack-years of smoking correlated with IMT thickness in the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) (41).

1.2.6.5 Blood Lipids
CIMT has been found to be significantly increased in patients with familial hypercholesterolemia (58, 59). The Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) showed greater progression of CIMT values over a 2-year period in men with high LDL cholesterol levels (41). Interventional studies with statin therapy, namely the Monitored Atherosclerosis Regression Study (MARS), Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) study, Regression Growth Evaluation Statin Study (REGRESS) have uniformly reported regression of IMT thickness with significant changes as early as six months (60-62). However in the Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE)
trial, ezetimibe, a cholesterol absorption inhibitor when combined with
simvastatin did not reduce the progression of IMT thickening as compared to
simvastatin alone despite reducing the LDL-C and CRP levels (63).

1.2.7 Association between carotid intima-media thickness and coronary
atherosclerosis

Many studies have shown carotid IMT to correlate with the severity of
coronary atherosclerosis. The Muscatine and Rotterdam coronary calcification
study (64, 65) found a significant correlation with computed tomography
coronary artery calcium score, even after adjusting for cardiovascular risk
factors. A coronary angiographic study found a strong correlation between
carotid IMT and coronary artery stenosis >50% luminal narrowing; if mean
IMT was more than 1.15 mm, patients had a 94% probability of having
significant CAD (66). Baldassarre et al found a significant correlation between
carotid IMT and coronary IMT values measured by intravascular ultrasound
(IVUS) of the coronary arteries, with correlation coefficients ranging from 0.49
to 0.55. A mean carotid IMT value of >1 mm was associated with a 7-fold
increased risk of having a significant coronary stenosis (minimal lumen area
<4mm²) by IVUS (67). Maximum and mean IMT values have also been found
to correlate significantly with the severity, extent and atheroma burden
assessed by quantitative coronary angiography (68). However the predictive
value of carotid IMT was weaker for proximal segments when compared to
mid and distal segments of the major coronary arteries. Lekakis et al found
that high IMT scores, estimated by incorporating data from carotid and
femoral arteries, correlated well with the extent of angiographic CAD and were
also predictive of multivessel disease (69). Kato et al found carotid IMT values were significantly higher in acute coronary syndrome patients with angiographically complex coronary plaques when compared to those with a solitary plaque (70).

As previously discussed in the pathologic process of atherosclerosis, the likely mechanism of association between increasing IMT and CAD is chronic inflammation leading to endothelial dysfunction and smooth muscle proliferation in the early stages, followed by lipid accumulation within the intra and extra cellular matrix, with further macrophage infiltration and more advanced lesions.

1.2.8 Carotid IMT in the prediction of cardiovascular events

In addition to correlating with past exposure to cardiovascular risk factors, CIMT is associated with prevalent cardiovascular disease and future cardiovascular risk. The relationship between CIMT and future CAD was first demonstrated in the KIHD study, in which, for every 0.1 mm increment of CIMT, the risk of future myocardial infarction increased by 11% (21). For CIMT values greater than 1mm, there was a two fold greater risk of acute myocardial infarction over a 3 year follow up period. The ARIC study (71) showed a prevalence of myocardial infarction in subjects in the highest quartile of CIMT of 5%. It was also noted that for every 0.19 mm increment in CIMT, the risk of death or myocardial infarction increased by 36%. The CAD risk was almost two fold greater in men with mean CIMT greater than 1 mm (22). In the Cardiovascular Health Study (CHS) the odds ratio for symptomatic CAD was 2.8 when the highest quartile of CIMT was compared with the
lowest quartile (47). The adjusted relative risk for myocardial infarction or stroke for the highest quintile with the highest IMT as compared with the lowest quintile was 3.8 in the follow up CHS study (30). In the Rotterdam study, a population based cohort study among subjects more than 55 years old, increased CIMT was associated with a risk of future cerebrovascular and cardiovascular events (24). A subsequent follow up study in this group of subjects found that carotid plaque and CIMT were strongly predictive of incident MI, even after adjusting for cardiovascular risk factors and medication use (72). CIMT is a reliable predictor of future events in subjects whose risk factor status is stable because it reflects the integrated effect of cumulative risk factor exposure. However, in subjects who are undergoing risk factor modification, it may not accurately reflect the disease burden. In these individuals, assessing CIMT progression might be a better indicator of future risk of cardiovascular events (73). In the Cholesterol Lowering Atherosclerosis Study (CLAS), it was found that an annual increase of IMT by 0.03 mm increases the risk of myocardial infarction 3-fold (74). In the meta analysis by Lorenz et al (75) from 8 observational studies representing 37,197 subjects with a mean follow up of 5.5 years carotid IMT was found to be a strong predictor of future vascular events. The age and sex adjusted relative risk of myocardial infarction was 1.26 and for stroke was 1.32 per 1-standard deviation difference in common carotid artery IMT. For an absolute carotid IMT difference of 0.1 mm, the future risk of MI increases by 10 to 15%, and the stroke risk increases by 13 to 18%.
1.2.9 Role in Primary and Secondary Prevention

1.2.9.1 Primary Prevention

The Framingham risk score (FRS) is currently the most established scoring system to assess cardiovascular risk. However, many subjects, particularly young adults are classified as low or intermediate risk despite the presence of multiple cardiovascular risk factors and metabolic syndrome based on FRS. It is well known that metabolic syndrome causes an increase in IMT and enhances progression of the atherosclerotic process (76). Thus in these subjects IMT measurement may be useful as an additional marker of vascular risk when making decisions to modify their cardiovascular risk. Baldassarre et al (77) have found that both FRS and CIMT are independent predictors of cardiovascular outcome in dyslipidaemic patients who are deemed to be at low or intermediate risk. The hazard ratio was estimated as 6.7 in patients with a FRS of 10-20% but with an elevated CIMT. These patients, who are not aggressively treated based on current guidelines, proved to have a risk similar to patients with FRS of 20 to 30%. The KIHD study in healthy Finnish middle-aged men showed that a carotid IMT of >1mm was associated with a two-fold greater risk of acute myocardial infarction over 3 years (78). The ARIC study in 45-64 year-old men and women has shown that a carotid IMT of 1mm or more was associated with an increased risk ratio of 2 to 5 for developing a coronary event over 4-7 years (22, 79). The CHS study and the Rotterdam study showed that increased CIMT was associated with a significantly greater risk for combined acute myocardial infarction and stroke (24, 30). All these studies are concordant in demonstrating that increased IMT is a powerful predictor of coronary and cerebrovascular complications.
1.2.9.2 Secondary prevention

In the CLAS study (80), it was shown that, in patients with established CAD, for each 0.03 mm increase per year in common carotid IMT, the relative risk for a coronary event was 3.1. In the Regression Growth Evaluation Statin Study (REGRESS) (62), which looked at the effect of Pravastatin on progression of IMT, in subjects with established CAD, a 0.05 mm annual reduction in mean carotid and femoral artery IMT reduced the absolute risk of cardiac events over a 2 year period by 10%. The Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) trial (61) found that treatment with Pravastatin in patients with CAD reversed progression of atherosclerosis and reduced IMT measurements during the 4 year follow up period. The Monitored Atherosclerosis Regression Study (MARS) (60) also showed similar results. These studies validate the use of carotid IMT as a surrogate marker of atherosclerosis and to estimate risk of future events. In the recently published Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) trial, CIMT was used as the surrogate marker for assessing progression of atherosclerosis and the results showed that the combined therapy of ezetimibe with simvastatin did not result in significant changes in CIMT values, as compared to simvastatin treatment alone despite decreased levels of LDL cholesterol and CRP (63).

1.2.10 Carotid plaque

1.2.10.1 Definition and types

Non obstructive plaque is readily visible with B-mode ultrasound scanning, with the best view of its encroachment into the lumen detected from the
transverse plane. In the Manheim carotid IMT consensus statement (38), plaque was defined as a focal structure encroaching the arterial lumen by at least 0.5 mm or >50% of the surrounding IMT value or a thickness of >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface. The most common location of plaque is the carotid bulb, followed by the internal carotid artery (ICA) and less commonly in the common carotid artery because of laminar blood flow (37).

Plaques are characterized as hyperechoic, isoechoic and hypoechoic, based on signal intensity. High intensity or hyperechoic signals are comparable with those detected from fascial layers or from adventitia of the artery and isoechoic signals are comparable to the signals from the muscles of the neck. The echogenicity of hypoechoic signals are similar to that of blood (81). Plaques have been characterized as homogeneous (i.e. of uniform echogenicity) or heterogeneous based on the echo texture of the plaque. Highly echogenic portions of heterogeneous plaque may represent calcification and echolucent areas may correspond to either lipid or haemorrhagic content. Various computer assisted systems are available to objectively measure carotid plaque echogenicity like gray scale medium (GSM) and integrated back scatter analysis.

Other aspects of non obstructive plaques, which have been studied, are plaque diameter (i.e. measuring the maximum incursion of the plaque into the lumen), the number of discrete plaques, and quantifying the number of segments of the carotid arteries containing plaque (82-84).
1.2.10.2 Prevalence of carotid plaque

The Cardiovascular Health Study (CHS) described the prevalence of extracranial carotid atherosclerosis in men and women aged over 65 years (47). Carotid atherosclerosis was detected in 75% of men and 62% of women, although the prevalence of >50% stenosis was only 7% in men and 5% in women. Maximum stenosis and maximum wall thickness increased with age and were uniformly greater at all ages in men than in women. Examination of 1,189 members of the Framingham cohort aged 66-93 years, revealed no disease in 30%, <50% stenosis in 62% and >50% stenosis in 8%, similar to the findings in CHS (85). In the Kuopio Ischemic Heart Disease (KIHD) study involving eastern Finnish men between 42-60 yrs of age, prevalence of detectable atherosclerotic plaques increased from 1% in those aged 42 years to 28% in those aged 60 years (86).

1.2.10.3 Vulnerable Plaque

Like carotid IMT, carotid plaque characteristics have been associated with stroke risk (87-89) and coronary events (90) in prospective studies. Several studies have shown an association between carotid plaque and traditional cardiovascular risk factors like hypertension, dyslipidaemia, smoking and diabetes (52, 91, 92). Vulnerable carotid plaque shares the same histologic features as observed in coronary arteries; namely, a thin fibrous cap, large lipid core, and high macrophage content (93, 94). Plaque instability is believed to be a systemic phenomenon and this hypothesis was tested in the European Carotid Surgery Trial, which found that patients with angiographically irregular carotid plaque surface are at increased risk of future AMI and sudden cardiac
death (95). A retrospective study done by Lombardo et al in patients scheduled for coronary artery bypass surgery found a significantly higher prevalence of complex carotid plaque (irregular surface and/or heterogeneous echogenicity) in patients with unstable angina than those with chronic stable angina (96). In a prospective study by Gronholdt et al, a 2-fold-higher risk of cardiac ischaemic events was found in patients with echolucent (complex) carotid plaques (89). In the Cardiovascular Health Study (CHS), patients with high risk, vulnerable plaque had a higher risk of CVD outcomes in age and sex adjusted analyses and complex plaques were more prevalent in patients with higher IMT values (97). Seo et al, in their study on patients with acute coronary syndrome (ACS), chronic stable angina and control subjects found a higher prevalence of echolucent plaques in ACS patients (98).

Contrast-enhanced ultrasound (CEUS) is a novel technique, with ability to directly visualise arterial vasa vasorum and intraplaque neovascularisation, thereby identifying vulnerable plaque. Studies have found good correlation between CEUS and histology in identifying neovascularisation within the plaque (99, 100). Investigators have also found more pronounced neovascularisation in echolucent plaques (101, 102). The presence and extent of adventitial and intraplaque neovascularisation has been correlated with prior cardiovascular events in an observational study (103). In a recent study by Deyama et al, higher grade neovascularisation of carotid plaque assessed by CEUS was significantly associated more complex and severe CAD (104).
1.2.11 Comparison of carotid and coronary plaque characteristics
The correlation between carotid and coronary plaques has been studied by several investigators. Autopsy studies done as early as 1962 by Mitchell et al found that patients with myocardial infarction had more stenosis and ulcerated plaques in the carotid arteries (105). Another study in asymptomatic hypercholesterolaemic patients who had a positive exercise test, found significant obstructive CAD by angiography in subjects who had carotid plaque demonstrated by ultrasonography (106). In the study by Kato et al (70) in ACS patients, those with multiple complex coronary plaques were found to have increased soft, echolucent plaques in the carotids along with an increase in CIMT measurements and positive carotid remodelling. Seo et al found a similar higher prevalence of echolucent plaques in ACS patients (98). The study by Sakaguchi et al found that multi vessel CAD on angiography was more often associated with significant carotid plaque than those with single vessel disease (107). Spence et al (108) have found that plaque area and plaque volume are closely associated with coronary events in that there is a 3-fold increase in the risk of MI in patients in the highest quartile of plaque area as compared to those in the lowest quartile.

1.2.12 Comparison of carotid intima media thickness and carotid plaque in the prediction of coronary artery disease and cardiovascular events
The accuracy of CIMT as a marker of atherosclerosis and as a tool to predict future cardiovascular events has been questioned by various research groups. The study by Lau et al found that carotid plaque was more prevalent in ACS patients compared to a control group, whereas their mean IMT score
was no different (109). A meta-analysis by Inaba et al of 11 population based studies (54,336 patients) comparing the diagnostic accuracies of carotid plaque and CIMT in predicting CAD events found that the 10-year myocardial infarction rates were significantly lower with a negative test result for carotid plaque compared with that of CIMT {[(4.0%; 95% CI 3.6–4.7%) vs (4.7%; 95% CI 4.2–5.5%)]} (110). In the study by Sakaguchi et al, carotid plaque score was equivalent to ICA-IMT measurements, but superior to CCA-IMT scores in predicting coronary artery disease (107).

1.3 Inflammation and atherosclerosis
Recent evidence has shown that atherosclerosis, rather than being an irreversibly progressive disease affecting the older population, is in fact a dynamic inflammatory process, which is potentially amenable to medical treatment. The link between inflammation and atherosclerosis has evolved at both the basic and clinical level over the past several years and this has helped to yield predictive and prognostic information of clinical importance.

Fatty streak, the earliest atherosclerotic lesion, which is quite common in infancy and early childhood, is an inflammatory lesion, consisting of monocyte derived macrophages and T-lymphocytes (111). The initial insult in this process is the injury to the vascular endothelium (112). Various triggering factors like elevated and oxidised LDL; free radicals as a result of smoking and other risk factors like diabetes and hypertension; genetic alterations; and various other factors lead to endothelial activation/dysfunction. This leads to increased expression of vasoactive molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and P-
selectin; proinflammatory cytokines like tumour necrosis factor (TNF), interleukin-1 (IL-1), interleukin-18 (IL-18); and growth factors like monocyte/macrophage colony stimulating factor (MCSF) and transforming growth factor β1 (TGF-β1). As a result, endothelial homoeostatic mechanisms alter, leading to increased adhesiveness and permeability of the endothelium to platelets and leukocytes (112). As this process continues, smooth muscle cells (SMC) migrate from the media into the intima and proliferate under the influence of growth factors, evolving into a more fibrotic plaque, which ultimately may create a stenosis (113).

Inflammation also contributes quite significantly to precipitate an acute thrombosis by destabilising the plaque (114). Inflammatory mediators block the creation of new collagen in the fibrous cap of the plaque and also cause destruction of existing collagen fibres (115). This makes the plaque prone to rupture, thereby exposing the arterial wall to thrombogenic material. This eventually leads to formation of thrombus, which can cause sudden obstruction to blood flow through the affected vessel (116).

1.3.1 Biomarkers of inflammation in atherosclerosis

Biomarkers of inflammation in atherosclerosis include acute phase reactants like C-reactive protein (CRP), fibrinogen, serum amyloid A (SAA) and plasminogen activator inhibitor 1 (PAI-1); cytokines like tumour necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-18 (IL-18); proteases such as matrix metalloproteinase (MMP-9); adipokines like adiponectin; adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1); platelet products like soluble CD40 ligand (sCD40L) and myeloid-related protein (MRP).
An ideal biomarker should have the following characteristics:

1. easy to measure
2. inexpensive
3. standardised assays with low variability
4. stability (no change with time of day, diet and from day to day)
5. provide independent cardiovascular risk estimation

1.3.1.1 CRP in cardiovascular disease

CRP is an acute phase reactant protein secreted predominantly by hepatocytes in response to cytokines like IL-6 and TNF during the inflammatory process (117). Recent studies have shown that it is also produced from the SMCs and endothelial cells of unobstructed coronary arteries (118, 119). CRP has been the most extensively studied biomarker of inflammation in cardiovascular disease and has proved to be robust, as it has standardised high sensitivity assays (hs-CRP), is a stable protein, has a long half-life and is easily measured (120). Traditional methods of measurement of CRP are designed for infectious disorders and typically estimate values between 3-5mg/L. However, high sensitivity assays measure values down to 0.3 mg/L, which is essential to risk stratify individuals in apparently healthy populations. The coefficient of variation of hs-CRP assays is generally <10% from the 0.3 to 10 mg/L range (121). The value that constitutes an elevation in serum hs-CRP is not clearly defined. The Centres for Disease Control and Prevention and American Heart Association (CDC/AHA) have defined values of <1, 1-3 and >3mg/L as low, average and high risk values for the determination of cardiovascular risk (122); these values approximate to the
corresponding tertiles in the general population. In patients with known coronary artery disease (CAD), a value of >3mg/L and >10mg/L are predictive of adverse outcome in stable CAD and in ACS respectively. Table 1.2 shows the conditions associated with increased and decreased levels of hs-CRP.

Data from multiple large-scale prospective studies have shown that CRP is an independent predictor of cardiovascular events like myocardial infarction, ischaemic stroke and sudden cardiac death (123-127). In the prospective study by Ridker et al, CRP was found to be a stronger predictor of risk than LDL cholesterol (127). CRP measurement also contributes to risk estimation in patients with metabolic syndrome (128) and at all levels of the Framingham score (125). A meta-analysis of prospective population based studies comparing persons in the lower tertile of hs-CRP with those of the upper tertile demonstrated an odds ratio of 2.0 (95% CI 1.6-2.5) for major coronary events (129). The Cholesterol and Recurrent Events (CARE) trial demonstrated that statin therapy lowers the CRP levels by 20 to 30% in addition to reduction of LDL levels (130). The Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial showed that in patients with CAD, intensive lipid lowering therapy resulted in a significant reduction in CRP and reduced atherosclerotic lesion progression (131). The Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE-IT TIMI 22) trial demonstrated similar associations between CRP reduction and risk of recurrent coronary events among patients with ACS (132). The GUSTO IV ACS Trial showed similar results in post ACS patients (133) The recently published results of the JUPITER (Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating
Rosuvastatin) trial, a prospective randomized placebo controlled study, which assessed the effect of statin therapy in apparently healthy individuals without hyperlipidaemia but with CRP >2mg/L, showed that statin therapy reduced CRP by up to 37% and also significantly reduced cardiovascular endpoints, including myocardial infarction, stroke, unstable angina and cardiovascular deaths (134).

The expert panel of CDC/AHA termed CRP as an independent marker of cardiovascular risk (122) and recommends the use of CRP as part of global risk prediction in asymptomatic individuals, especially subjects who are classified as intermediate risk for cardiovascular disease by conventional risk factors. The recommended cut off points in clinical practice are CRP concentrations <1 mg/L for low-risk and >3 mg/L for high-risk individuals. However, the panel have indicated that data on African, South Asian and Native American descent populations, who may be at higher risk of CVD, are limited.

**Table 1.2. Conditions associated with increased and decreased levels of hs-CRP.**

<table>
<thead>
<tr>
<th>Increased Levels</th>
<th>Decreased Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raised Blood pressure</td>
<td>Moderate alcohol consumption</td>
</tr>
<tr>
<td>Increased BMI</td>
<td>Physical activity/endurance exercise</td>
</tr>
<tr>
<td>Smoking</td>
<td>Weight loss</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>Statins/Fibrates/Niacin</td>
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<tr>
<td>Diabetes Mellitus</td>
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<tr>
<td>Low HDL/high Triglycerides</td>
<td></td>
</tr>
<tr>
<td>Oestrogen/progesterone use</td>
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<tr>
<td>Chronic infections/Inflammatory</td>
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<td>disorders</td>
<td></td>
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</tbody>
</table>

Pearson et al (122)
1.3.1.2 Fibrinogen

Fibrinogen, the precursor of fibrin, is the major coagulant protein, and an important determinant of blood viscosity and platelet aggregation (135, 136). A high plasma level of fibrinogen is associated with increased cardiovascular risk (137). Several prospective epidemiologic studies have demonstrated a positive association between the risk of CAD and fibrinogen levels and have also shown that baseline fibrinogen levels predict future risk of myocardial infarction and stroke (138-141). When compared to CRP, fibrinogen is a less potent predictor of cardiovascular events (142). However, if a reliable, high-quality immunoassay is used to measure the levels, there is good correlation between fibrinogen and CRP levels and it is predictive of future cardiovascular events (143).

The relationship between fibrinogen and carotid IMT has been studied in several population based observational studies. A meta-analysis of these studies by Baldassarre et al found that CIMT values had unequivocal association with fibrinogen and CRP levels (144).

1.3.1.3 Osteopontin

Osteopontin (OPN) is a novel molecule with proinflammatory functions and is a potent inhibitor of vascular calcification. It is a glycosylated protein and in human adults, it is generally restricted to bone, kidneys and epithelial linings. Plasma levels of OPN have been associated with various inflammatory conditions, including cardiovascular disease. OPN has been shown to be expressed in animal and human atherosclerotic lesions, especially associated with macrophages and foam cells (145, 146). OPN levels have been found to
correlate with extent of CAD in some studies (147, 148). In a small, cross sectional study done by Coskun et al, OPN levels were significantly elevated in the ACS patients compared to stable CAD and control groups, but there was no correlation with the severity of CAD (149).

1.3.1.4 Paraoxonase-1
Paraoxonase-1 (PON1) is a HDL-associated protein contributing to the anti-inflammatory and antioxidant properties of HDL. In animal experiments, PON1 knock out mice fed with atherogenic diet showed acceleration of the atherosclerotic process and over expression of PON1 in mouse models led to reduced oxidative stress and reduction in atherosclerotic lesion size (150, 151). In the Caerphilly study, PON1 activity was 20% lower in subjects who had a new coronary event (152). Bhattacharya et al found that subjects with PON1 activity in the highest quartile had significantly lower cardiovascular event rates (153). In a small observational study, subjects with high HDL, but low PON1 levels were found to be more susceptible to CAD than those with low HDL and high PON1 levels (154). Recently, a meta analysis of 43 studies (20,629 subjects) found that decreasing levels of PON1 activity was an independent risk factor for CAD (155).

1.3.1.5 Apo A1
Apolipoprotein A1 (Apo A1) is the main component of HDL cholesterol and constitutes about 60% of the polypeptide content. It plays a crucial role in the reverse cholesterol transport and also has anti-inflammatory and anti oxidant properties. It prevents lipid peroxidation and formation of oxidized LDL.
Studies have found low levels of Apo A1 in acute myocardial infarction patients even after adjustment for baseline characteristics and cholesterol levels (156-158). Another study found that Apo A1 was better than HDL-C to predict presence of angiographic coronary artery disease (159). Apo A1 was also found in a study to be able to predict the severity of CAD compared to HDL cholesterol (160).

1.4 Left ventricular remodelling and geometry

1.4.1 Left ventricular remodeling and geometry in CAD

Left ventricular remodelling is a dynamic process characterised by changes in the myocellular and extracellular matrix compartments of the myocardium (161). This may be an adaptive change during normal physiologic growth or could be pathological because of either acute or chronic mechanical stresses like myocardial infarction, hypertension or valvular heart disease (162). Three different patterns of ventricular remodelling are recognised as shown in figure 1.4 based on the mechanical stresses involved and include:

1. Pressure overload- a typical clinical example is aortic stenosis and this leads to concentric hypertrophy where the myocytes thicken and the left ventricular mass increases without chamber dilatation.

2. Volume overload- (e.g. aortic regurgitation) this leads to eccentric hypertrophy characterised by stretching of myocytes and chamber dilatation.

3. Post infarct remodelling- Stretched and dilated infarcted myocardium increases LV volume, with volume and pressure load on non infarcted areas.
Concentric left-ventricular hypertrophy, when a pressure load leads to growth in cardiomyocyte thickness (dotted lines represent left ventricle growing inwards); eccentric hypertrophy, when a volume load produces myocyte lengthening; and post-infarct, when the stretched and dilated infarcted tissue increases the left-ventricular volume with a combined volume and pressure load on the non-infarcted zones (dotted lines represent combined effects of concentric and eccentric hypertrophy). Fibrosis contributes to all three patterns, Opie et al, (161).

The LV remodelling changes alter the LV geometry in different ways and this has been extensively studied. Echocardiographic techniques have been used to delineate the different alterations in LV geometry. M-mode echocardiographic LV end diastolic dimension (LVEDD), end-diastolic thickness of the interventricular septum (IVST) and posterior wall thickness
(PWT) are obtained using a leading edge technique (163) and LV mass (LVM) and relative wall thickness (RWT) are calculated as follows:

\[ \text{LVM} (g) = 0.8[1.04 (\text{LVEDD} + \text{IVST} + \text{PWT})^3 - (\text{LVEDD})^3] + 0.6; \]

\[ \text{RWT} = \frac{(\text{IVST} + \text{PWT})}{\text{LVEDD}}. \]

Four patterns of LV geometry have been well characterised based on LVM and RWT (164) as depicted in figure 1.3. They are as follows:

1. Normal- Normal LV mass and normal RWT
2. Concentric remodelling- Normal LV mass with increased RWT
3. Eccentric hypertrophy- Increased LV mass with normal RWT
4. Concentric hypertrophy- Increased LV mass and Increased RWT

Left ventricular hypertrophy is a strong predictor of adverse prognosis in CAD (165, 166). Various studies have assessed the association of the different geometric patterns on cardiovascular events and mortality. Koren et al reported that LV mass and geometry were useful in risk stratification of patients with hypertension, and also that concentric hypertrophy was more strongly associated with cardiovascular events, including myocardial infarction (167). Ghali et al prospectively studied 988 predominantly African American patients who underwent both coronary angiography and echocardiography for presumed CAD for a mean follow up period of 9 years and reported that the concentric hypertrophy group had the highest all-cause and cardiovascular mortality, eccentric hypertrophy moderately increased the risk of death and there was no significant increase in mortality with concentric remodelling (168). In the Framingham Heart Study, subjects with concentric hypertrophy had the worst prognosis, followed by eccentric hypertrophy, concentric
remodelling and normal geometry. However, when the values were adjusted for differences in LV mass, the association was attenuated (169). In the Jackson cohort of the ARIC study involving African Americans, it was shown that concentric hypertrophy was associated with diastolic dysfunction, eccentric hypertrophy was associated with systolic dysfunction and that concentric remodelling was not associated with either systolic or diastolic dysfunction (170).

**Fig 1.5: Patterns of LV geometry**

<table>
<thead>
<tr>
<th>Relative Wall Thickness</th>
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<tr>
<td>Normal</td>
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<td>Increased</td>
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<tr>
<td>Normal</td>
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<tr>
<td>Normal</td>
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<tr>
<td>Concentric remodeling</td>
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<tr>
<td>Increased</td>
</tr>
<tr>
<td>Eccentric Hypertrophy</td>
</tr>
<tr>
<td>Concentric Hypertrophy</td>
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</table>

Modified from Sehgal et al, (171)

In the Coronary Artery Risk Development in Young Adults (CARDIA) study, coronary artery calcium (CAC) score by computed tomography (CT) was positively associated with left ventricular mass (172). In the Losartan Intervention For End point reduction in hypertension (LIFE) study, it was found
that patients with CAD had greater LV mass and larger cavity dimensions compared to the non-CAD group (173).

1.4.2 LV geometry and CIMT

The relationship between carotid IMT and LV geometry and remodeling patterns have been studied and reported in hypertensive subjects and also in general population. The Assessment of Prognostic Risk Observational Survey (APROS) prospectively studied 1142 untreated hypertensive subjects with ultrasound examinations of the heart and carotid arteries. It was found that there was a positive relationship between LV mass and CIMT values, and that patients with concentric LVH had the highest levels of CIMT values, followed by concentric remodelling, eccentric hypertrophy and normal geometry (174).

In the study by Di Bello et al of asymptomatic, untreated hypertensive subjects, carotid IMT was found to be significantly correlated with LV mass, r=0.35 (175). Moreover, in this study, they also found that, age and systolic blood pressure were the only independent predictors of IMT and LVM. Similar finding was found in a population based study where the prevalence of hypertension was not significantly different between the four groups of LV geometry (176). Carotid IMT was found to be independently associated with relative wall thickness (RWT) in a study of hypertensive subjects by Park et al (177). A study on apparently healthy individuals found good correlation (r=0.37, P <0.01) between arterial stiffness index measured by pulse wave analysis and CIMT values (178).

A possible mechanism of the association between CIMT and LV mass and geometry is parallel blood pressure dependent changes of the cardiac and
vascular structure. The elevated BP often leads to decreased arterial compliance and increased afterload of the heart leading to compensatory hypertrophy. Also, the smooth muscle cells in the intima media complex are susceptible to pressure overload and undergo hypertrophy/hyperplasia and this reflects as increased CIMT. Another mechanism is likely to be blood pressure independent and possibly related to age, leading to an adaptive response to local distending pressure, pulsatile load and shear stress inducing intrinsic alterations in the arteries. Moreover, non haemodynamic factors including neurohumoral and genetic factors also might play a role in the cardiac and vascular remodeling patterns.

1.5 Summary
Carotid IMT has been found to be a valid marker of subclinical atherosclerosis and as a predictor of future cardiovascular events in observational and epidemiological studies. It is being increasingly used as a surrogate marker in clinical trials assessing the effects of treatments for CAD. It has the potential to be used in both primary and secondary prevention of CAD. However, questions have been raised over its utility in assessing the extent of atherosclerosis. Moreover, carotid plaque has been found to correlate better than IMT measurements to predict cardiovascular events and extent of CAD. The methodologies in the estimation of IMT have considerably varied in the studies done so far, in that numerous studies have included plaque measurements in their IMT. Also, in earlier studies, imaging was done with relatively low resolution scanners and the CIMT were taken as single point measurements with calipers. These issues have been addressed in the
recently published Mannheim consensus statement, which has suggested a standardised protocol for image acquisition and measurement of CIMT and to differentiate between plaque and IMT when reporting results. Also, the availability of sophisticated software for image analysis has improved the reproducibility of CIMT measurements. A similar strategy has been developed for studying carotid plaque characteristics, which has helped to identify vulnerable plaques. Increasing numbers of biomarkers are being tested to identify early atherosclerosis and also to risk stratify and predict future cardiovascular events in patients at risk. Hs-CRP has been accepted as a biomarker for use in clinical practice and results from several other biomarkers like fibrinogen, osteopontin and paraxonase 1 are promising. Left ventricular geometric patterns have been studied extensively in hypertensive patients; however, there is limited data available on the relation between left ventricular mass and CIMT in non hypertensive group of patients and virtually none looking at correlation between CIMT and LV mass in the wide spectrum of CAD. A multi marker approach combining the established inflammatory marker hs-CRP with a structural vascular marker like CIMT and novel biochemical and vascular markers of cardiovascular disease may offer additive prognostic information for adverse outcomes.

1.6 Research Hypotheses

• Carotid plaque is a better predictor of coronary artery disease compared to CIMT
• Carotid intima media thickness is marker of arterial remodelling and not atherosclerosis
• Carotid plaque burden correlates with the severity of coronary artery disease
• Assessment of carotid plaque morphology might be helpful to identify vulnerable coronary plaque and patients at risk of developing ACS.
• Left ventricular geometry and LV mass correlate with CIMT.
• Assessment of left ventricular diastolic function may be useful to differentiate those with unobstructed coronary arteries from those with obstructive coronary artery disease.
• A combination of novel biomarkers may be useful to identify patients at risk of developing acute coronary syndromes.

We aim to assess the carotid ultrasound markers in a wide spectrum of CAD patients, namely those with unobstructed coronary arteries, stable CAD and ACS. We will be following standardised protocol for the measurement of carotid intima-media thickness as suggested by the Mannheim Consensus statement (measuring CIMT in the common carotid artery in a plaque free region) and also to assess the plaque score and morphology to confirm the association with angiographic coronary artery disease and identifying a vulnerable plaque. We also aim to compare CIMT and plaque to find out a better predictor of CAD and if a composite score consisting of CIMT and plaque will be a better marker for these patients in predicting CAD. We postulate CIMT as remodelling process and not a true reflection of atherosclerosis and aim to compare this with cardiac remodelling pattern, namely LV geometry. We aim to assess left ventricular systolic and diastolic function using tissue doppler imaging technique to see if it could add additional value in predicting obstructive CAD. Novel serum biomarkers may
be useful as a screening tool in patients at risk of developing a cardiovascular event and we aim to perform biomarkers involving different pathways of atherosclerosis.
CHAPTER 2
Chapter 2. Objectives and methods

2.1 Introduction
In this section, the study objectives are discussed followed by the methodology, data collection, observer variability testing and a discussion about statistical methods.

2.2 Objectives
The main objectives of this study were as follows:
(i) To compare carotid plaque extent, severity and characteristics in patients with chest pain and non obstructive coronary arteries, chronic stable angina and acute coronary syndromes.
(ii) To determine the carotid ultrasonographic markers that best predict angiographic coronary artery disease.
(iii) To evaluate the relationship between left ventricular geometry, carotid atherosclerosis, serum biomarkers and coronary artery disease.

2.3 Study Design
This was a prospective, observational, cross sectional study conducted in a tertiary care centre. All study procedures were carried out at a single site - The Manchester Heart Centre. The Central Manchester University Hospitals NHS Foundation Trust acted as the sponsor of the research project.

2.4 Study Population
The study population consisted of patients who underwent coronary
angiography at the Manchester Heart Centre as part of investigation or treatment of chest pain between June 2009 and January 2011. These patients were either referred by their primary care physicians to the rapid access chest pain clinics (RACPC) for investigation of stable chest pain which was presumed to be of cardiac origin or patients who were admitted with acute chest pain and diagnosed with an acute coronary syndrome, thereby requiring in-patient coronary angiography with a view to revascularization as appropriate. Only those patients presenting with acute chest pain for the first time (without any previous history of ACS) were included in the study. The study population was racially diverse, reflecting the local population of central Manchester. Participants were divided into 3 groups as follows:

(i) Group 1: those with unobstructed coronary arteries (No-CAD)

(ii) Group 2: those with chronic stable angina and proven angiographic coronary artery disease (>50% stenosis in at least one coronary artery)

(iii) Group 3: those with an acute coronary syndrome and proven angiographic coronary artery disease (>50% stenosis in at least one coronary artery)

2.5 Ethical Approval

A completed ethics application was submitted to the Cumbria and Lancashire B Ethics Committee on 30/01/2009. After attending the committee meeting on 12/02/2009, and making appropriate amendments in the participant information sheet and informed consent form, ethical approval was given on
10\textsuperscript{th} March 2009. Following this, the Research and Development (R&D) at the Central Manchester University Hospitals NHS Foundation Trust (CMFT) gave approval on 15\textsuperscript{th} May 2009.

2.6 Patient Recruitment

2.6.1 Inclusion criteria

(i) Provision of signed informed consent

(ii) Patients undergoing coronary angiography for investigation or management of coronary artery disease

2.6.2 Exclusion criteria

(i) Inadequate carotid ultrasonographic or echocardiographic images

(ii) History of high-grade carotid artery stenosis or occlusion

(iii) History of carotid endarterectomy or carotid stenting

(iv) Previous history of an acute coronary syndrome (unstable angina and myocardial infarction)

(v) Previous coronary artery by-pass graft surgery

(vi) History of primary myocardial disease e.g. dilated cardiomyopathy, viral myocarditis, hypertrophic cardiomyopathy

(vii) History of or echocardiographic evidence of significant valvular disease (more than mild valvular regurgitation and any degree of left ventricular outflow tract obstruction or valvular stenosis)

(viii) Any history or current cardiac arrhythmias e.g. atrial fibrillation.
2.6.3 Screening and Enrolment

Patients who underwent coronary angiography for the investigation of chest pain or an acute coronary syndrome were asked to participate in the study. All participants were required to give written informed consent. Recruitment took place over a period of 18 months.

Patients were recruited from the Manchester Heart Centre, located in Central Manchester University Hospitals NHS Foundation Trust. The list of patients undergoing elective coronary angiography was obtained from the cardiac catheter lab database, ‘CARDEX’. Participant information sheets were given to them during their pre-admission clinic visit and they were contacted through telephone to find out if they were willing to participate in the study and also to clarify any questions regarding the study procedures and the conduct of the study. All eligible and willing participants were then recruited for the study. Patients admitted with possible acute coronary syndrome were approached during their hospital stay in acute admissions unit (MAU) and in acute cardiology wards and were given the participant information sheet. All eligible and willing participants were recruited into the study and they had their study procedures performed either during the index hospital admission or they were given an appointment to come back to the research clinic within 2 weeks to have the study procedures performed.
As shown in Figure 2.1, 1326 patients were screened for eligibility and 792 were excluded as they did not meet the inclusion criteria. A further 321 patients could not be recruited as they were transferred from other peripheral hospitals for urgent cardiac catheterisation and returned to their respective hospitals on the same day. A total of 213 patients were enrolled into the study out of which 35 patients refused to participate and 32 patients either had inadequate images or had other significant cardiac pathologies. A total of 146 patients were recruited into the study.
2.7 Study procedures

2.7.1 General Clinical Assessment

All participants recruited into the study underwent a full history and physical examination including assessment of coronary risk factors.

2.7.2 History

The definitions of CAD risk factors used were:

- Hypertension: ≥140 mmHg systolic blood pressure and/or ≥90 mm Hg diastolic blood pressure readings or current anti-hypertensive therapy
- Hypercholesterolemia: total cholesterol >6.2 mmol/l or LDL cholesterol >4.0 or receiving current cholesterol lowering therapy
- Family history of cardiovascular disease: MI, angina or sudden death in a first degree relative (male<55 years, female<65 years)
- Diabetes Mellitus: fasting plasma glucose>7.0mmol/L or current hypoglycaemic therapy
- Smoking: Smoking history including number of cigarettes per day and number of years of smoking were documented. Those participants who quit smoking > 1 year ago were considered as ex-smokers.

Patients were ascribed as having chronic stable angina or an acute coronary syndrome (unstable angina/ non ST elevation myocardial infarction/ ST elevation myocardial infarction) based on the following definitions:

- Chronic stable angina: chest pain with exertion and relieved by rest or glyceryl trinitrate (GTN), suggestive of myocardial ischaemia.
- Unstable angina: angina of increasing frequency or severity, occurring
on minimal exertion or at rest

- Non ST elevation myocardial infarction (NSTEMI): NSTEMI was defined by electrocardiographic (ECG) ST-segment depression or prominent T-wave inversion and positive biomarker of necrosis (troponin) in the absence of ST-segment elevation and in an appropriate clinical setting (chest discomfort or anginal equivalent).

- ST-elevation myocardial infarction (STEMI): STEMI was defined by electrocardiographic ST segment elevation ≥1 mm in at least 2 standard leads or ≥2 mm in at least 2 contiguous precordial leads and positive biomarker of necrosis (troponin) with persistent chest pain >20 minutes.

2.7.3 Physical examination

All participants had a focused cardiovascular physical examination during the visit to the research clinic. Heart rate and blood pressure were measured during the study visit. Blood pressure was measured by the conventional auscultatory technique using a stethoscope and sphygmomanometer. The subject was allowed 5-10 minutes of semi-reclined rest in a warm environment. The arm was exposed and an appropriate sized cuff was applied. The arm was supported in a horizontal position and the diaphragm of the stethoscope was placed over the brachial artery. The cuff was inflated to a level above the estimated systolic pressure at which auscultatory sounds could not be heard and the pressure was gradually reduced. The point of onset of auscultatory sounds was taken as the systolic blood pressure and the disappearance of auscultatory sounds as the diastolic blood pressure. In
those with elevated blood pressure readings, a second recording was obtained after 1-2 minutes and the average of these two was taken as the blood pressure reading.

A cardiovascular examination was carried out including careful auscultation over the chest to see if there were any significant murmurs suggestive of valvular heart disease or evidence of heart failure.

2.7.4 Anthropometric assessment

All participants underwent anthropometric assessment during the study visit. Height, weight, waist circumference were measured.

Participants were asked to stand bare feet against the scale to measure their height and it was measured in centimetres.

A standard weighing scale, which was calibrated periodically, was used to measure the weight. The same scale was used for all the study participants in the study. Weight was measured in kilograms.

Waist circumference was measured with a heavy-duty inelastic fiberglass tape that was calibrated against a metal tape measure to ensure accuracy. The subjects stood with legs parallel and shoulder-width apart. Waist circumference was measured at mid distance from last rib margin and iliac crest as shown in figure 2.2, at the end of normal expiration as recommended by the NCEP-ATP III guidelines. Circumferences were taken with the tape placed directly on the skin (not over clothing).
Body mass index was then calculated using the standard formula:

\[ \text{BMI} = \frac{\text{Weight (Kg)}}{(\text{Height in meters})^2}. \]

2.7.5 Laboratory assessments and serum biomarkers

Laboratory investigations were performed in the University of Manchester Lipid Laboratory. Blood samples were obtained during the study visit. The patients were requested to attend the research clinic after having fasted for a period of 12 hours. Blood samples were obtained from the antecubital vein using an 18-gauge needle and vacutainer and collected into EDTA tubes (for lipids and biomarkers), citrate (for fibrinogen), serum (for paraoxonase and biomarkers) and fluoride-oxalate (for glucose). The samples were then allowed to stand for an hour. Plasma and serum samples were then isolated by centrifugation at 1500 x g for 15 min at 4°C. Aliquots (0.5 ml) of serum and plasma were stored at -80°C for up to 6 months before assays were performed.
The samples were then analysed in batches at the Cardiovascular research Laboratories, University of Manchester under the supervision of Dr Valentine Charlton-Menys by experienced laboratory technicians. Advice was sought from expert in the field of biomarker analysis (Dr Handrean Soran) about the biomarker assays to be done in the study and it was decided to perform a combination of established biomarkers and novel biomarkers which were least likely to be affected by medications (e.g., statins). Cholesterol was measured using the CHOD-PAP method, triglycerides by the GPO-PAP method, HDL cholesterol was assayed using a second generation homogenous direct method. LDL cholesterol was calculated using the Friedewald equation. Apolipoproteins Al (apo AI, Randox Laboratories, Dublin, Ireland) was assayed using immunoturbidimetric assays with a Cobas Mira analyser (Horiba ABX Diagnostics, Nottingham, UK). The laboratory participated in the RIQAS (Randox International Quality Assessment Scheme), which is a rigorous scheme for external quality assessment (EQA). CRP and osteopontin were assayed in plasma using DuoSet® ELISA development kits from R&D Systems, Abingdon, UK. Serum paraoxonase activity (PON1) was determined using a semi-automated microtitre plate method (Charlton-Menys V, Clin Chem 2006;52:453-457). Fibrinogen was assayed in citrated plasma using the method of Jacobsson. Fibrinogen was clotted with thrombin and fibrin was synergised on glass beads before removal of other plasma proteins by washing using water and centrifugation. Fibrin was solubilised in alkaline-urea solution (urea 6 mol/l in sodium hydroxide 0.2 mol/l in water) before determination of absorbance at 300 nm. Fibrinogen concentration was calculated using absorbance readings obtained with the WHO International
Standard: 2nd International Standard for Fibrinogen Plasma, obtained from NIBSC, South Mimms, Herts, UK. Results were adjusted for dilution of plasma citrate solution (x1.22).

2.7.6 Carotid Ultrasonography

2.7.6.1 Imaging Technique

Carotid ultrasonography was performed by a single operator (Dr Satheesh Nair) using a high-resolution ultrasound system equipped with a 10 MHz linear array transducer. The spatial resolution of this transducer was 0.1 mm.

Carotid ultrasonography was performed with the patient in the supine position, the shoulders and head resting on a pillow and the head turned to the opposite direction of the side to be scanned. The patients were connected to a 3 lead ECG and traces were optimised to achieve a good ‘R’ wave voltage. Both carotid arteries were examined in a systematic manner obtaining transverse and longitudinal views of the common carotid arteries (CCA), carotid bulbs, internal (ICA) and external carotid arteries (ECA). Most patients were scanned at a standard depth of 4 cm, but for those patients with large neck or deeper vessels, the depth was adjusted to acquire optimal images with a frame rate of >25 Hz.

The scanning procedure was started on the right side of the neck, in the transverse axis, initially at the lower portion of the neck in order to identify the common carotid artery. The transducer was then slowly moved upwards to the angle of the mandible, identifying the carotid bulb, the bifurcation and the internal and external carotid arteries. The images with at least 3-5 cardiac
cycles to include all the above-mentioned segments of the carotid system were then stored digitally as a cine-loop. The longitudinal axis of the carotid system was then obtained by rotating the transducer by 90 degrees. After visualising the common carotid artery, fine adjustments were made to the transducer position and image settings to obtain the double line appearance of the intima-media complex in the far wall of the distal common carotid artery, adjacent to the bifurcation. The image with clearly visualised bifurcation and the distal CCA was then stored digitally to include at least three cardiac cycles.

The carotid system was then screened for presence of atherosclerotic plaque. Plaque was defined as a focal structure encroaching into the arterial lumen by more than 50% of the surrounding wall or a thickness more than 1.5 mm as suggested by the Mannheim consensus statement. The images were stored digitally onto the archive system and analysed off-line for measurements of carotid intima-media thickness and plaque characteristics.

2.7.6.2 Carotid IMT measurement

Carotid IMT was measured using the automated edge detection software package (GE echopac). The IMT was measured at the far wall of the common carotid artery, 1cm proximal to the carotid bulb for a length of 1cm as shown in figure 2.3. The software analysed 300 points within this 1cm and gave a minimal, maximal and average IMT scores respectively. IMT was measured at end diastole (R wave on the ECG trace) as per recommended guidelines. Three sets of readings on different cardiac cycles were obtained on each side and then averaged.
2.7.6.3 Carotid plaque assessment

Plaque was defined as a focal structure encroaching into the arterial lumen by more than 50% of the surrounding wall or a thickness more than 1.5 mm. An example of carotid plaque is shown in figure 2.4. The number of plaques was counted in the proximal and distal CCA, carotid bulb, ICA and ECA to give the total number of plaques. To derive the plaque score, the carotid system was divided into 5 segments on each side, the proximal CCA, distal CCA (distal 2 cm proximal to the carotid bulb), the carotid bulb, the proximal ICA and the proximal ECA. A score of 1 was given to each segment with a plaque. Hence the maximum plaque score on each side would be 5. The plaque scores on
both sides were then added up together to give a score out of 10. Plaque morphology was analysed based on the gray scale appearance of the plaque. The gain setting was standardised by adjusting the time gain compensation to achieve a noiseless vessel lumen area and echogenic adventitial layer. Those plaques, which were bright (white), were classed as echodense and those, which were translucent (grey), were classed as echolucent and those, which had features of both as mixed.

2.7.6.4 Carotid remodelling assessment

Lumen diameters and inter-adventitial diameters of the common carotid arteries were measured. Lumen diameter was measured as the distance between the far and near wall intimal layers. The inter-adventitial diameter was measured as the distance between the near wall inter-adventitial interface to far wall inter-adventitial interface. Lumen diameter and intima-media thickness was used to measure arterial wall cross-sectional area, also referred to as vascular mass using the following formula:

$$\text{CSA} = \pi (\text{IMT} + D/2)^2 - \pi (D/2)^2$$

IMT is intima media thickness, D is lumen diameter and CSA is cross-sectional area.

2.7.7 Echocardiography

2.7.7.1 Image acquisition and techniques

Echocardiography was performed by a single operator (SN) using a commercially available machine (GE Vivid 7 Dimension) with a 2-3 MHz broadband transducer. The examination was performed with the patient in the
left lateral decubitus position. The images were stored digitally into an archive system for off-line analysis.

**Figure 2.4: Example of carotid plaque in the carotid bulb region.**

Conventional parasternal and apical views were acquired. The long axis plane runs parallel to the left ventricle as shown in figure 2.5, the short axis is perpendicular to the long axis, and the 4-chamber plane is orthogonal to the other two. The 2-chamber view was obtained by 90 degrees counter-clockwise rotation from the position of the 4-chamber view. M-mode, 2-D, colour flow and Doppler imaging techniques were used to obtain the required echocardiographic parameters.

The transducer was placed in the right hand of the operator and firstly positioned along the left sternal border to obtain the left parasternal long and short axis views. The apical 4-chamber and 2-chamber views were then acquired by placing the transducer over the cardiac apex.
Figure 2.5: Standard image planes for two-dimensional trans thoracic echocardiographic imaging.

A- Long-axis plane, B- four-chamber plane and C- short-axis plane.

2.7.7.2 M-mode measurements

M-mode measurements of end-diastolic septal thickness, posterior wall thickness and cavity diameter were derived from the short-axis parasternal view at the mid cavity level to calculate left ventricular mass and categorize left ventricular geometry.

M-mode measurements were made according to the recommendations of the American Society of Echocardiography. Left ventricular mass index was calculated using the modified Devereux formula as follows:

$$LVMI = 0.8 \times (1.04 \times \left( [LVID + PWT + IVST]^3 - [LVID]^3 \right)) + 0.6 \text{ g divided by body surface area.}$$

LVMI refers to left ventricular mass index, LVID to left ventricular internal
dimension, PWT to posterior wall thickness and IVST to interventricular septal thickness. The presence or absence of left ventricular hypertrophy was based on a left ventricular mass index cut-off of 115 g/m$^2$ for men and 95 g/m$^2$ for women.

Relative wall thickness was derived from the formula $2 \times \text{PWT}/\text{LVID}$. The pattern of left ventricular geometry was classified on the basis of the presence or absence of left ventricular hypertrophy and a relative wall thickness cut-off point of 0.42, into one of four categories as follows:

1. Normal = No LVH + RWT ≤ 0.42
2. Concentric remodelling = No LVH + RWT > 0.42
3. Eccentric hypertrophy = LVH + RWT ≤ 0.42
4. Concentric hypertrophy = LVH + RWT > 0.42

**2.7.7.3 Two-dimensional assessment**

Left ventricular end-systolic and end-diastolic volumes and ejection fraction were calculated from the apical 4-chamber and 2-chamber views using the modified Simpson’s biplane method as shown in figure 2.6. Left atrial dimension was obtained from the parasternal long axis view at end-systole and left atrial area and volumes were estimated form the apical 4-chamber and 2-chamber views. The LA volume was then indexed to body surface area to derive the LA volume index (LAVI).
Figure 2.6: 2D assessment of LV ejection fraction by biplane modified Simpson’s method.

2.7.7.4 Doppler Imaging

2.7.7.4.1 Pulsed Wave Doppler Imaging

Pulsed wave Doppler measurements of trans-mitral and left ventricular outflow velocities were obtained. Normal trans mitral flow is of low velocity. The early diastolic velocity (E wave) and a late diastolic velocity (A wave) were measured as shown in figure 2.7 and the E/A ratio was used as an estimate of LV diastolic function. The normal value of E/A ratio varies with age and a value of >1 is considered normal in those less than 60 years of age. Also, using this Doppler recording, deceleration time (DT), defined as the interval from the peak of E wave to the baseline along the deceleration slope was measured. The flow in the left ventricular outflow tract (LVOT) was
interrogated using a sample 1 cm below the aortic valve. Pulsed sample was also obtained to include both aortic and mitral valve Doppler traces and this was used to measure isovolumic relaxation time (IVRT), the LV ejection time (ET) and the mitral valve closure to opening time (MCO). These measurements were used to calculate the myocardial performance index (Tei index), an index, which combines both systolic and diastolic cardiac function.

The formula is as follows:

\[(\text{MCO} - \text{ET})/ \text{ET} \] where MCO = mitral valve closure to opening time, ET = ejection time.

The normal value is 0.39 ± 0.05 for the LV.

**2.7.7.4.2 Tissue Doppler Imaging**

Tissue Doppler imaging was performed from the apical 4- chamber view with a sample volume (2 to 3 mm in length) positioned in the myocardium along the basal ventricular wall, about 1 cm from the mitral annulus. Recordings were made at end-expiration during normal quiet respiration. Signals were recorded from 4 left ventricular walls, namely, septal, lateral, anterior and inferior walls in the apical 4 and 2 chamber views. The pattern of myocardial motion is similar, but inverted and lower in velocity, compared to trans mitral flow velocities. There is a peak systolic velocity (S wave), an early diastolic velocity (e’) and a late diastolic velocity (a’) as shown in figure 2.8. The E/e’ ratio (E velocity from trans mitral pulsed Doppler to e’ velocity by TDI) was calculated and these measurements were used to assess stroke volume, regional long-axis systolic function and global and regional diastolic function. The averaged values of the peak systolic and early diastolic velocities and the
the averaged E/e’ were also analysed and compared between study groups.

Figure 2.7 Pulsed wave doppler trace across the mitral valve.

E = early diastolic velocity and A = late diastolic velocity.

Colour M-mode technique was performed in the apical 4-chamber view by placing the M-mode cursor parallel to the mitral valve. The flow propagation velocity was measured by the slope along a distinct isovelocity (aliasing) line from the mitral valve plane into the LV cavity. The normal value is > 40 cm/sec. This technique is a helpful tool in detecting LV diastolic dysfunction.

2.7.7.5 Grading of diastolic function

Left ventricular diastolic function was graded as per the British Society of Echocardiography recommendations and the diastolic parameters obtained from pulsed wave and tissue doppler were included in the calculation as shown in table 2.1. Diastolic function was graded into 4 different categories
namely, normal diastolic function, mild, moderate and severe diastolic dysfunction.

**Figure 2.8 Pulsed tissue Doppler trace across the lateral mitral annulus.**

S= peak systolic tissue velocity, e’= early diastolic tissue velocity and a’= late diastolic tissue velocity.

**Table 2.1 Grading of LV diastolic function**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>E/A ratio</td>
<td>1-2</td>
<td>&lt;1</td>
<td>1-2</td>
<td>&gt;2</td>
</tr>
<tr>
<td>IVRT (msec)</td>
<td>50-100</td>
<td>&gt;100</td>
<td>50-100</td>
<td>&lt;50</td>
</tr>
<tr>
<td>DT (msec)</td>
<td>150-200</td>
<td>&gt;200</td>
<td>150-200</td>
<td>&lt;150</td>
</tr>
<tr>
<td>e’/a’</td>
<td>1-2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;&lt;1</td>
</tr>
<tr>
<td>E/e’</td>
<td>&lt;8</td>
<td>-</td>
<td>-</td>
<td>&gt;13</td>
</tr>
</tbody>
</table>
2.7.8 Reproducibility Testing

Intra and inter observer variability on both carotid and echocardiographic parameters were studied. The operator (SN) who performed all the study scans randomly selected 10 patients and the measurements were repeated and this was analysed for intra observer variability. For a previous multi centre study, the operator (SN) had performed two sets of carotid scans on each of the ten volunteers with an interval of at least a week between the two scans. This was digitally archived and analysed by an independent operator remotely for intra observer variability and obtained a certificate of accreditation (Appendix VIII). Also, an analysis for inter observer variability was also performed. An independent operator who was blinded to the study participant information and only had access to the images performed this. This operator was very experienced imaging physician and was very familiar with the study protocol and study parameters measured. This independent operator randomly selected 10 image sets and their carotid and echocardiographic measurements were repeated. This was analysed for inter observer variability.

2.7.8.1 Intra and inter observer variability of carotid Ultrasound measurements

The table 2.2 shows the intra and inter observer variability of different carotid ultrasound measurements done on 10 randomly selected study subjects from the entire study population. The variability was expressed as coefficient of variation. The intraobserver variability of carotid measurements ranged form 2.4% to 4.7% and inter observer variability was in the range of 3.6 to 9.7%. A
systematic review by Kanters et al, of 23 studies that reported intra and inter observer variability found intraobserver variability between 2.5% to 10.6% and inter observer variability was 3.1% to 18.3% in different studies. The mean IMT from common carotid artery was found to have the best reproducibility (34).

**Table 2.2: Intra and interobserver variability of carotid measurements**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intra observer variability CV (%)</th>
<th>Inter observer variability CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum IMT</td>
<td>3.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Mean IMT</td>
<td>2.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Max IMT</td>
<td>3.0</td>
<td>5.3</td>
</tr>
<tr>
<td>IMT-max</td>
<td>4.7</td>
<td>8.1</td>
</tr>
<tr>
<td>Plaque Score</td>
<td>2.4</td>
<td>9.7</td>
</tr>
</tbody>
</table>

CV = coefficient of variation

**2.7.8.2 Intra and inter observer variability of echocardiographic measurements**

The table 2.3 shows the intra and inter observer variability of echocardiographic measurements done on 10 randomly selected study subjects. The intra observer variability was 1.8% to 5.1% for different measurements. The inter observer variability was 2.1 to 11.5%. Overall, the reproducibility was best for tissue Doppler measurements in our study. The interobserver variability for LV ejection fraction estimation by biplane method was reported by 3-9% in a study (179). In another study, the inter observer variability for pulsed wave and tissue Doppler measurements were reported to have a variability of about 2-5% (180). Cho et al, reported an intraobserver
and interobserver variability for global circumferential strain as 4.9% and 6.3%, respectively (181).

Table 2.3: Intra and interobserver variability of echocardiographic measurements.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intra observer variability CV (%)</th>
<th>Inter observer variability CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass</td>
<td>4.7</td>
<td>8.8</td>
</tr>
<tr>
<td>RWT</td>
<td>4.3</td>
<td>9.0</td>
</tr>
<tr>
<td>LVEF</td>
<td>4.2</td>
<td>6.6</td>
</tr>
<tr>
<td>E/A</td>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td>Averaged E/e’</td>
<td>2.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Averaged e’</td>
<td>1.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Averaged s’</td>
<td>4.3</td>
<td>9.8</td>
</tr>
<tr>
<td>GCS</td>
<td>5.1</td>
<td>7.1</td>
</tr>
<tr>
<td>LAV</td>
<td>4.1</td>
<td>9.3</td>
</tr>
<tr>
<td>TAPSE</td>
<td>5.1</td>
<td>11.5</td>
</tr>
</tbody>
</table>

CV = coefficient of variation.

2.7.9 Angiographic data analysis

All the angiogram images were analysed by an experienced, independent operator who did not have access to patient details. The images were identified by the study ID number. The coronary tree was analysed visually in a very systematic manner to identify lesions. Significant coronary artery disease was defined as presence of ≥50% stenosis in the artery. An example of significant coronary stenosis is shown in figure 2.10.

2.7.9.1 Syntax scoring

Syntax score is a unique angiographic tool to assess the complexity of coronary artery disease. The score was originally tested in a randomised trial
(Syntax trial) of patients with multi vessel/Left main stem coronary artery disease comparing the outcomes of percutaneous coronary intervention versus coronary artery bypass surgery. It is a semi-quantitative, visual assessment of coronary vasculature from the angiographic images. This scoring system not only takes into account the number and severity of lesions but also the complexity of individual lesions. Coronary lesions of diameter stenosis ≥ 50% in vessels ≥ 1.5 mm must be included in the score. The coronary arterial tree is divided into 16 segments and significant lesions in the segments are given a score based the usual blood flow to the left ventricle from that artery or segment. For example, a significant lesion in the left main stem artery is given a score of 5, whereas a lesion in the diagonal branch scores 0.5. A multiplication factor of 2 is applied for non-occlusive lesions (50-99%) and a factor of 5 for total occlusions. Other lesion characteristics which score additional points include aorto-ostial location, bifurcation/trifurcation lesions, vessel tortuosity, calcification, presence of thrombus and lesion length of >20 mm. The total syntax score is derived from summing up all the individual scores of the lesions. Based on the total score, the coronary lesions are classified as low score <0-22, intermediate 23-32 and high score ≥33.

2.7.10 Statistical Analysis

Statistical advice was obtained from the study statistician and the sample size was decided based on this. It was anticipated that the cohort will consist of 15% normal, 25% chronic stable angina and 60% ACS. The sample size was determined by the number of referrals within the practical study period. As this is an exploratory study, formal power calculations were difficult and not
informative. One often used rule of thumb is that there needs to be a minimum of 10 events for each variable considered, although experience suggests that twice this number is required to avoid over fitting. The study statistician supervised data analysis. Standard statistical methods for medical research were adopted for data analysis. Kolmogorov-Smirnov test was done to determine if the data followed a normal distribution. Continuous variables

Figure 2.9: Angiographic view showing severe right coronary artery stenosis
were expressed either as means ± standard deviations or medians and interquartile ranges. Categorical variables were presented as proportions (percentages). T-test was used to compare the means of continuous variables between two groups and for more than 2 groups, one-way analysis of variance (ANOVA) was used. For non-normally distributed data, non-parametric test in the form of Mann-Whitney U test was used to compare between 2 groups and Kruskall Wallis test for more than 2 groups. Chi-square test or Fisher’s exact test were used for categorical variables to test the differences between groups. Spearman’s rank correlation analysis was performed to find out the correlation between variables. Receiver-operating characteristic (ROC) curves were constructed and the areas under the curves were used to determine the predictability of the test. A P-value of <0.05 was considered to be significant to reject the null hypothesis. Advice was sought from the study Statistician regarding performing a multivariate analysis using regression models and it was felt that there were not adequate events (plaques) in the groups to test the independent variables, and hence we opted to perform a correlation analysis. All calculations were done using SPSS 20.0 for Mac statistical program (SPSS Inc, Chicago, IL, USA).
CHAPTER 3
Chapter 3. Results

3.1 Demographic Characteristics of Study Population

3.1.1 Introduction
A total of 146 participants were recruited into the study from June 2009 to January 2011. The baseline demographic and clinical characteristics of the study population are described below.

3.1.2 Baseline Characteristics of Study Population
The baseline characteristics of the study population are shown in the table 3.1 below. The mean age of the study population was 56.9 ± 10.6 (range 29 to 85) years of whom 82% were men. Of these, 80% were white, 16% were of south Asian origin and 4% were of African Caribbean origin. Approximately half of the study group had history of hypertension, 25% had diabetes and about two thirds had hyperlipidaemia, smoking history and a family history of coronary artery disease. A very high proportion of subjects were already established on aspirin (90%) and statin (88%) therapy. The majority were also on beta-blocker (68%) and ACE inhibitor (55%) therapy.

3.1.3 Anthropometric and clinical assessment
The height, weight and waist circumference were measured for the study population and body mass index (BMI) and body surface area (BSA) were calculated using standard formulae and the mean values are shown in the table 3.2 below. As shown in Figure 3.1, only 27.4% of the study population had a BMI < 25 kg/m2, i.e. ideal weight category. 41.1% were overweight (BMI 25 to 29.9 kg/m2) and 31.5% were obese (BMI ≥ 30 kg/m2).
between genders showed that 70.1% of men and 80.8% of women were either overweight or obese (p = 0.39). The systolic blood pressure was
elevated (>140 mm Hg) in 18.6% and the diastolic blood pressure was > 90 mm Hg in 4% of the subjects.

### Table 3.2 Anthropometric and clinical assessments of study population

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>172.3± 8.8</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>83.4± 15.3</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>100.8± 10.4</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>28.1± 4.4</td>
</tr>
<tr>
<td>BSA (m$^2$)</td>
<td>2.0± 0.2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>67.7 ± 12.5</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>124.8 ± 17.0</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>72.1 ± 10.2</td>
</tr>
</tbody>
</table>

3.1.4 Baseline Blood results

The table 3.3 shows the baseline blood results of the study population. Ten percent of the patients had evidence of renal impairment (eGFR < 60), 8% had mild anaemia and none had values less than 10.0 g/dl. Forty-nine percent had abnormal fasting glucose levels: 20% in the impaired fasting glucose (IFG) range (6-7 mmol/L) and 29% had values in the diabetic range (>7.0 mmol/L). Based on the WHO definition of hypercholesterolaemia (≥6.2 mmol/l), 11% had raised values, whereas if cut off values were lowered to 5.2 mmol/l, 30% had raised cholesterol levels. 17% had triglyceride values.
values >2.5 mmol/l and 32% had values <1.0 mmol/l HDL-C levels.

3.2 Coronary Angiographic Findings

All 146 patients recruited in the study had undergone coronary angiography. 89 (61%) of these had presented with stable chest pain symptoms. They were either seen in the Rapid Access Chest Pain Clinic (RACPC) or in the cardiology outpatient clinics. The decision to perform coronary angiography was based on a full clinical evaluation and either results of exercise ECG or functional imaging. Fifty-seven (39%) patients presented with acute onset cardiac sounding chest pain. As per protocol, these patients did not have a previous history of coronary artery disease (CAD) and all underwent coronary angiography during the index admission. Out of the 89 patients presenting with stable chest pain, 30% of patients had unobstructed coronary arteries, 37% of patients had single vessel CAD, 24% of patients had 2-vessel disease and 9% of patients had triple vessel disease. In those with single vessel
disease, the left anterior descending artery (LAD) was the culprit vessel in 45%, left circumflex system (LCx) in 15% of patients and the right coronary artery (RCA) in 39% of patients. In those with 2-vessel disease, 10% had left main stem (LMS) disease, 29% had LAD and LCx involvement, 47% had LAD and RCA disease and 14% had LCx and RCA stenoses.

**Table 3.3: Baseline blood results of study population**

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (µ mol/l)</td>
<td>91.5 ± 68.1</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td></td>
</tr>
<tr>
<td>≥ 90</td>
<td>46.9</td>
</tr>
<tr>
<td>60-89</td>
<td>42.8</td>
</tr>
<tr>
<td>30-59</td>
<td>6.2</td>
</tr>
<tr>
<td>15-29</td>
<td>2.1</td>
</tr>
<tr>
<td>&lt;15</td>
<td>2.1</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>14.0 ± 1.5</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.0 ± 2.2</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/l)</td>
<td>4.5 ± 1.3</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.2 ± 0.3</td>
</tr>
</tbody>
</table>

Out of the 57 patients who presented with acute chest pain, 34 patients had presented with ST-elevation myocardial infarction (STEMI), 19 had presented with non ST-elevation acute coronary syndrome (NSTEMI) 16 with non ST-elevation myocardial infarction (NSTEMI) and 3 patients had unstable angina.
On coronary angiography, 4 patients were found to have unobstructed coronary arteries and 53 patients had significant coronary stenoses. Of the latter 53 patients, 60% had single vessel disease, 21% had 2-vessel disease and 19% patients had triple vessel disease. In the acute coronary syndrome (ACS) patients with single vessel disease, 56% had LAD stenosis, 9% had LCx disease, 31% had RCA involvement and 3% had intermediate arteries stenosed. In those with 2-vessel disease, 9% had LMS disease, 55% had LAD with RCA involvement, 27% had LCx with RCA disease and 9% had LAD with intermediate artery involvement.

The study participants were divided into groups based on the angiographic findings as follows:

- Unobstructed coronary arteries (No-CAD)- 31 patients
- Obstructive coronary artery disease (CAD)- 115 patients

The CAD group was further divided into those with

- Stable CAD- 62 patients
- Acute coronary syndrome (ACS)-53 patients

### 3.3 Comparison of baseline characteristics

In the following section, the comparative analysis of the baseline characteristics of different groups are discussed. Table 3.4 shows a comparison of the baseline clinical characteristics of those with and without
Table 3.4 Comparison of baseline variables in those with and without obstructive CAD

<table>
<thead>
<tr>
<th></th>
<th>No-CAD (n=31)</th>
<th>CAD (n=115)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male) %</td>
<td>74.2</td>
<td>84.3</td>
<td>0.19</td>
</tr>
<tr>
<td>Mean Age (yrs)</td>
<td>53.5 ± 10.1</td>
<td>57.9 ± 10.6</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>80.6</td>
<td>80.0</td>
<td>0.69</td>
</tr>
<tr>
<td>Asian</td>
<td>12.9</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>6.5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>38.7</td>
<td>48.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>19.4</td>
<td>27.0</td>
<td>0.39</td>
</tr>
<tr>
<td>Hypercholesterolaemia (%)</td>
<td>77.4</td>
<td>70.4</td>
<td>0.44</td>
</tr>
<tr>
<td>Smoking Hx (%)</td>
<td>32.3</td>
<td>40.9</td>
<td>0.45</td>
</tr>
<tr>
<td>Family Hx (%)</td>
<td>71.0</td>
<td>66.1</td>
<td>0.61</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>69.0 ± 11.3</td>
<td>67.4 ± 12.9</td>
<td>0.53</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>128.0 ± 17.4</td>
<td>123.9 ± 16.9</td>
<td>0.23</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>74.6 ± 10.3</td>
<td>71.3 ± 10.1</td>
<td>0.12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.8 ± 4.8</td>
<td>27.6 ± 4.1</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.9 ± 3.4</td>
<td>7.3 ± 3.4</td>
<td>0.57</td>
</tr>
<tr>
<td>eGFR ≥ 60 (%)</td>
<td>87.1</td>
<td>90.4</td>
<td>0.08</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>14.0 ± 1.4</td>
<td>13.9 ± 1.5</td>
<td>0.72</td>
</tr>
</tbody>
</table>

obstructive CAD. Student t test was used to compare the means of the two groups. All baseline characteristics were comparable between the groups except that those with CAD were significantly older and those in the No-CAD group had higher BMI.
Table 3.5: Comparison of baseline variables between stable CAD and ACS

<table>
<thead>
<tr>
<th></th>
<th>Stable CAD (N= 62)</th>
<th>ACS (N= 53)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (% male)</td>
<td>82.3</td>
<td>86.8</td>
<td>0.61</td>
</tr>
<tr>
<td>Mean Age (yrs)</td>
<td>58.8± 10.2</td>
<td>56.8± 11.0</td>
<td>0.33</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>74.2</td>
<td>86.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Asian</td>
<td>24.2</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>50.0</td>
<td>47.2</td>
<td>0.85</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>30.6</td>
<td>22.6</td>
<td>0.40</td>
</tr>
<tr>
<td>Hyperlipidaemia (%)</td>
<td>87.1</td>
<td>50.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking Hx (%)</td>
<td>32.3</td>
<td>50.9</td>
<td>0.13</td>
</tr>
<tr>
<td>Family Hx (%)</td>
<td>67.7</td>
<td>64.2</td>
<td>0.70</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>67.3 ± 14.1</td>
<td>67.4 ± 11.4</td>
<td>0.96</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>125.7 ± 17.4</td>
<td>121.8 ± 16.1</td>
<td>0.22</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>70.2 ± 10.4</td>
<td>72.8 ± 9.8</td>
<td>0.17</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.6 ± 4.3</td>
<td>26.4 ± 3.6</td>
<td>0.005</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>7.2 ± 3.4</td>
<td>7.6 ± 3.5</td>
<td>0.53</td>
</tr>
<tr>
<td>eGFR ≥ 60 (%)</td>
<td>87.1</td>
<td>94.2</td>
<td>0.08</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.8 ± 1.5</td>
<td>14.1 ± 1.6</td>
<td>0.24</td>
</tr>
</tbody>
</table>
Table 3.5 shows a comparison between patients with stable CAD and ACS. Student t-test was performed to compare the means of the two groups. There was a significant difference in the ethnicity distribution between the groups, in that, there were more white individuals and less South Asians in the ACS group compared to the stable CAD group. There were also a significantly higher proportion of patients with hyperlipidaemia in the stable CAD group compared to the ACS group (87% vs. 51%) and they had a higher BMI. Other baseline variables were comparable between the groups.

Table 3.6 shows the comparative analysis of demographic variables between the 3 groups of patients, namely, those with unobstructed coronary arteries by angiography, those with stable CAD and those with ACS. The Kruskall Wallis statistical test was performed to compare between the groups and a p value of <0.05 implies that the difference in mean between the groups is statistically significant. There was a significant difference between the groups in the proportion of patients with hyperlipidaemia and smoking, in that, the history of hyperlipidaemia was most prevalent amongst those with stable CAD and least in ACS group of patients and history of smoking was significantly higher in the ACS patients. There was significant difference in the BMI between the 3 groups. A significantly higher proportion of patients with stable CAD and ACS were on preventative medications. Other baseline characteristics were comparable between the groups.
Table 3.6 Comparison of demographic and clinical variables between those with No-CAD, stable CAD and ACS.

<table>
<thead>
<tr>
<th></th>
<th>No-CAD (31/146)</th>
<th>Stable CAD (62/146)</th>
<th>ACS (53/146)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male)%</td>
<td>74.2</td>
<td>82.3</td>
<td>86.8</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean Age (yrs)</td>
<td>53.5 ± 10.1</td>
<td>58.8 ± 10.2</td>
<td>56.8 ± 11.0</td>
<td>0.07</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>80.6</td>
<td>74.2</td>
<td>86.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Asian</td>
<td>12.9</td>
<td>24.2</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>38.7</td>
<td>50.0</td>
<td>47.2</td>
<td>0.54</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>19.4</td>
<td>30.6</td>
<td>22.6</td>
<td>0.91</td>
</tr>
<tr>
<td>Hyperlipidaemia (%)</td>
<td>77.4</td>
<td>87.1</td>
<td>50.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoking Hx (%)</td>
<td>32.3</td>
<td>32.3</td>
<td>50.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Family Hx (%)</td>
<td>71.0</td>
<td>67.7</td>
<td>64.2</td>
<td>0.51</td>
</tr>
<tr>
<td>Medications (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>73.3</td>
<td>95.2</td>
<td>92.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Statin</td>
<td>75.0</td>
<td>93.4</td>
<td>94.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>50.0</td>
<td>66.1</td>
<td>82.7</td>
<td>0.007</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>33.3</td>
<td>41.9</td>
<td>86.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>69.0 ± 11.3</td>
<td>67.2 ± 14.1</td>
<td>67.4 ± 11.4</td>
<td>0.82</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>128.0 ± 17.4</td>
<td>125.7 ± 17.4</td>
<td>121.8 ± 16.1</td>
<td>0.23</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>74.6 ± 10.3</td>
<td>70.2 ± 10.4</td>
<td>72.8 ± 9.8</td>
<td>0.12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.8 ± 4.8</td>
<td>28.5 ± 4.3</td>
<td>26.4 ± 3.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.9 ± 3.4</td>
<td>7.2 ± 3.4</td>
<td>7.6 ± 3.5</td>
<td>0.70</td>
</tr>
<tr>
<td>eGFR ≥ 60 (%)</td>
<td>87.1</td>
<td>87.1</td>
<td>94.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>14.0 ± 1.4</td>
<td>13.8 ± 1.5</td>
<td>14.1 ± 1.6</td>
<td>0.46</td>
</tr>
</tbody>
</table>

3.4 Carotid Ultrasound Findings

The results of the carotid IMT and plaque measurements are described in the following sections.

3.4.1 Carotid intima-media thickness measurements
The carotid ultrasound findings are summarized in table 3.7. Maximal IMT with plaque (IMT-max) was calculated by including the maximal plaque diameter in those patients with plaque and the mean value was 1.83± 1.05 mm. The distribution of the IMT values was categorized into three groups as shown in table 3.8, namely <0.5 mm, 0.5-1.0 mm and >1.0 mm. More than half of study subjects had min IMT < 0.5 mm, around 90% had their mean and Max IMT in the 0.5-1.0 mm category and the IMT-max score was > 1.0 mm in around two-thirds of the subjects.

**Table 3.7: Carotid Ultrasound findings in the study population.**

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min IMT (mm)</td>
<td>0.51± 0.12</td>
</tr>
<tr>
<td>Mean IMT (mm)</td>
<td>0.67± 0.14</td>
</tr>
<tr>
<td>Max IMT (mm)*</td>
<td>0.83± 0.17</td>
</tr>
<tr>
<td>IMT-max (mm)**</td>
<td>1.83± 1.05</td>
</tr>
<tr>
<td>Plaque number (median)</td>
<td>1.0</td>
</tr>
<tr>
<td>Plaque Score (median)</td>
<td>1.0</td>
</tr>
<tr>
<td>Plaque Echogenicity (%)</td>
<td></td>
</tr>
<tr>
<td>Echodense</td>
<td>40.6</td>
</tr>
<tr>
<td>Echolucent</td>
<td>28.7</td>
</tr>
<tr>
<td>Mixed</td>
<td>30.7</td>
</tr>
<tr>
<td>Vascular Mass (cm²)</td>
<td>0.15± 0.04</td>
</tr>
</tbody>
</table>

*Max IMT (Maximal IMT thickness),

**IMT-max (Intima media thickness including plaque)

### 3.4.2 Carotid plaque assessment

Carotid plaque was present in 59% of the study population. Fifty-seven percent of the plaques were seen in the carotid bulb, 10% in the common carotid artery, 6% in the internal carotid artery and 27% were found in multiple
carotid arterial segments. The plaques were echodense in 41%, echolucent in 29% and of mixed echogenecity in 30% of the study population. The mean plaque thickness was $2.2 \pm 0.8$ mm. The median plaque score of the study population was 1.0; inter quartile range of 0.0-2.0 and a maximum score of 7.0.

**Table 3.8: IMT measurements categorized into tertiles**

<table>
<thead>
<tr>
<th>IMT (mm)</th>
<th>&lt; 0.5</th>
<th>0.5-1.0</th>
<th>&gt;1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min IMT (%)</td>
<td>53.8</td>
<td>45.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Mean IMT (%)</td>
<td>6.9</td>
<td>90.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Max IMT* (%)</td>
<td>0.0</td>
<td>86.9</td>
<td>13.1</td>
</tr>
<tr>
<td>IMT-max** (%)</td>
<td>0.0</td>
<td>33.8</td>
<td>66.2</td>
</tr>
</tbody>
</table>

*Max IMT (Maximal IMT thickness),
**IMT-max (Intima media thickness including plaque)

### 3.4.3 Vascular mass calculation

Vascular mass of the carotid artery was calculated as described in the methodology chapter. The mean vascular mass of the study population was $0.15 \pm 0.04$ cm$^2$.

### 3.5. Echocardiographic Findings

#### 3.5.1 Left ventricular chamber quantification and geometry

The left ventricular dimensions of the study population are shown in table 3.9. Sixteen percent of subjects had increased IVSd dimension. Less than 1% of
men had raised LVEDD compared to 8% of women. Nine percent of subjects had increased PWd. The left ventricular mass index, calculated using the modified Devereux formula was raised in 24% and 46% of men and women, respectively. In the study population, 51% had normal geometry, 30% had concentric remodelling, 9% had eccentric hypertrophy and 10% had concentric hypertrophy.

**Table 3.9 Left ventricular chamber quantification**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSd (cm)</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>LVEDD (cm)</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>PWd (cm)</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>LVMi (g/m²)</td>
<td>90.1 ± 23.7</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>86.7 ± 23.1</td>
</tr>
<tr>
<td>LVESV (ml)</td>
<td>30.1 ± 14.4</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>65.5 ± 8.7</td>
</tr>
</tbody>
</table>

**3.5.2. LV Volumes and systolic function assessment**

LV volume assessment was done from apical 4-chamber and 2-chamber views using the biplane modified Simpson’s method. The results are shown in table 3.9. Twenty-nine percent of subjects had raised LVEDV and 9% had elevated LVESV amongst the study population. 89.5% of the study population had normal LVEF. 7% had mild LV impairment (EF 45-54%), 2.8% had moderate impairment (EF 36-44%) and 0.7% had severe LV systolic dysfunction (EF≤35%).
3.5.3 Diastolic function assessment

3.5.3.1 LV inflow Doppler

The mean E/A ratio of the study population was 1.0± 0.3 (normal range = 1-2); 49% had E/A ratio between 1-2 and 50% had <1 and 1% had E/A ratio >2. The mean isovolumic relaxation time (IVRT) was 93.7± 15.4 msec (normal range = 50-100). In 68% of patients, it was within the normal range, and in 32% of subjects, it was >100 msec. The mean deceleration time (DT) was 207.1± 42.0 msec (normal range = 150-200), 42% had normal values. In 5%, DT was <150 msec and in 53% of subjects it was >200 msec.

3.5.3.2 Mitral Annular Tissue Doppler Imaging

Pulsed wave tissue Doppler imaging (TDI) performed in the apical 4- chamber and 2- chamber views showed the following findings as shown in table 3.10. The mean septal e’ velocity was 7.7± 2.0 cm/s (normal value ≥ 8 cm/s), mean lateral e’ velocity was 10.2± 3.4 cm/s (≥ 10 cm/s), mean inferior e’ velocity was 8.6± 2.7 cm/s and mean anterior e’ velocity was 8.9± 1.9 cm/s. The normal values for inferior and anterior e’ velocities have not been established. The ratio of mitral E velocity to e’ velocity (E/e’) is shown in table 3.10 for septal, lateral, inferior and anterior walls. A value of <8 is considered to be associated with normal LV filling pressures and a value of >15 is suggestive of elevated filling pressures. However, there is no clear consensus as to which wall measurement should be used. The distribution of E/e’ values were divided into 3 categories as shown in table 3.11. The septal E/e’ was normal (<8) in 26%, whereas from the lateral wall, it was 58%. Majority of the subjects
fell into the intermediate category (8-15) except in the lateral wall and only a small proportion had values of >15 in all the 4 LV walls.

**Table 3.10 Myocardial tissue Doppler velocities in LV walls.**

<table>
<thead>
<tr>
<th>Mean Velocities</th>
<th>S (cm/s)</th>
<th>e’ (cm/s)</th>
<th>a’ (cm/s)</th>
<th>E/e’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septal</td>
<td>7.9± 1.5</td>
<td>7.7± 2.0</td>
<td>9.7± 1.9</td>
<td>10.1± 3.2</td>
</tr>
<tr>
<td>Lateral</td>
<td>8.7± 2.3</td>
<td>10.2± 3.4</td>
<td>9.6± 2.3</td>
<td>7.9± 2.6</td>
</tr>
<tr>
<td>Inferior</td>
<td>8.5± 1.8</td>
<td>8.6± 2.7</td>
<td>10.5± 2.3</td>
<td>9.3± 3.3</td>
</tr>
<tr>
<td>Anterior</td>
<td>7.8± 2.0</td>
<td>8.9± 3.0</td>
<td>8.9± 1.9</td>
<td>9.0± 3.0</td>
</tr>
</tbody>
</table>

**Table 3.11 Categories of E/e' values in different LV walls.**

<table>
<thead>
<tr>
<th>E/e’</th>
<th>&lt;8</th>
<th>8-15</th>
<th>&gt;15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septal (%)</td>
<td>25.6</td>
<td>67.6</td>
<td>6.8</td>
</tr>
<tr>
<td>Lateral (%)</td>
<td>57.9</td>
<td>41.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Inferior (%)</td>
<td>36.4</td>
<td>59.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Anterior (%)</td>
<td>41.4</td>
<td>55.0</td>
<td>3.6</td>
</tr>
</tbody>
</table>

3.5.3.3 Left atrial assessment

The left atrial diameter was measured in the parasternal long axis view and the mean value for the study population was 3.6± 0.5 cm (normal range =3.0-4.0 cm in men and 2.7- 3.8 cm in women). 20% of men and 23% women had increased left atrial diameter. The LA area and volume were measured in the apical 2-chamber and 4-chamber views. The mean LA area in this study was
16.9± 3.8 cm$^2$ (normal value is <20 cm$^2$) and mean LA volume (LAV) was 45.4± 15.1 ml (normal range = 18-58 ml in men and 22-52 ml in women). 23% of men and 27% of women had increased LA volume. The mean LA volume index (LAVI) was 22.3 ± 7.5 ml/m$^2$ (22.1 ± 6.6 ml/m$^2$ in men and 23.3 ± 10.8 ml/m$^2$ in women). The normal range is 16-28 ml/m$^2$. 78% had normal LAVI, 15% had mild LA enlargement, 5% had moderate and 2% had severely dilated LA.

3.5.3.4 Grading of diastolic function
LV diastolic function was graded as per the British Society of Echocardiography guidelines as previously mentioned in the methodology chapter. 50% of the subjects had abnormal diastolic function (44% mild, 6% moderate and none had severe diastolic dysfunction).

3.5.4. Myocardial performance index
The myocardial performance index (Tei index) showed a mean value of 0.39 ± 0.17 in the study. 79% had normal MPI (< 0.50).

3.6 Serum biomarker results
The results of the blood biomarkers are summarized in table 3.12. CRP values showed a wide range with an IQR of 1.2 to 11.9.
Table 3.12 Biomarker results in the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean± SD/ Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/l)</td>
<td>3.2 (1.2-11.9)</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.9± 0.8</td>
</tr>
<tr>
<td>Apo A1 (g/l)</td>
<td>1.6± 0.3</td>
</tr>
<tr>
<td>Osteopontin (ng/ml)</td>
<td>65.4 (30.1- 96.8)</td>
</tr>
<tr>
<td>PON 1 (nmol/ml/min)</td>
<td>86.8 (34.3- 135.9)</td>
</tr>
</tbody>
</table>

3.7 Summary of carotid ultrasound, echocardiographic and serum biomarker findings in the study population

Carotid IMT values, when divided into tertiles were distributed as follows: 90% of the subjects had mean IMT values between 0.5-1.0 mm (intermediate tertile), whereas the IMT-max (including plaque) was in the highest tertile (>1.0 mm) in two-thirds of patients. Carotid plaque was demonstrated in 69% of the patients and the median plaque score was 1. The plaque morphology was spread between echodense, echolucent and mixed echogenicity with slight predominance of echodense plaques.

LVMI was raised in 24% of men and 46% of women in the study. 51% had normal LV geometry, 30%, 9% and 10% each had concentric remodelling, eccentric hypertrophy and concentric hypertrophy, respectively. Around 90% of the study population had normal LV systolic function by EF estimation. 50% of the subjects had abnormal diastolic function and most of them had mild degree of dysfunction. Tissue Doppler imaging showed that the mean septal peak systolic velocity (Sm) was reduced amongst the study group and the septal E/e’ was in the normal range (<8) only in a quarter of patients. About
20-25% of the subjects had abnormal LA dimensions in the form of increased LA diameter, LA volume or LA volume index. Left ventricular myocardial performance index (Tei index), which is considered to an indicator of both systolic and diastolic function was normal in 79% of the subjects. The wide range of the hs-CRP values in this study reflects the study population ranging from unobstructed coronaries to those with acute coronary syndrome.

Further discussion about the study findings in relation to different groups of patients will be done in the following sections.

### 3.8 Comparison of Carotid Ultrasound findings

#### 3.8.1 Comparison of Carotid Ultrasound findings in No-CAD vs. Stable CAD vs. ACS

The comparative analysis of the carotid ultrasound findings of the 3 groups was performed using the Kruskall Wallis statistical test and a p value of <0.05 implies that the difference in mean between the groups is statistically significant. The results are shown in the table 3.13. There was no significant difference between the groups in minimal IMT, mean IMT and maximal IMT. However, when plaque was included in the IMT measurement (IMT-max), it was significantly different, in that those in the stable CAD and ACS groups had much higher values. Figure 3.2 shows the box plot of the distribution of IMT values in the 3 groups. Similarly, plaque score was significantly higher in both stable CAD and ACS groups compared to the unobstructed coronaries group. The plaque echogenicity was not significantly different between the
groups although there was a trend towards more echogenic plaques in the stable CAD group and more echolucent plaques in the ACS group. The vascular mass was not significantly different between the groups.

Table 3.13: Comparative analysis of the carotid ultrasound findings in unobstructed coronaries, stable CAD and ACS groups.

<table>
<thead>
<tr>
<th></th>
<th>No-CAD (n=31)</th>
<th>Stable CAD (n=62)</th>
<th>ACS (n=53)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min IMT (mm)</td>
<td>0.50±0.13</td>
<td>0.50±0.11</td>
<td>0.53±0.13</td>
<td>0.41</td>
</tr>
<tr>
<td>Mean IMT (mm)</td>
<td>0.64±0.16</td>
<td>0.67±0.13</td>
<td>0.69±0.15</td>
<td>0.25</td>
</tr>
<tr>
<td>Max IMT (mm)</td>
<td>0.80±0.18</td>
<td>0.83±0.15</td>
<td>0.85±0.18</td>
<td>0.30</td>
</tr>
<tr>
<td>IMT-max (mm)</td>
<td>1.24±0.58</td>
<td>1.85±1.00</td>
<td>2.17±1.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Presence of plaque (%)</td>
<td>45.2</td>
<td>75.8</td>
<td>75.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Plaque Score (median)</td>
<td>0.0</td>
<td>2.0</td>
<td>2.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Plaque Morphology (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echodense</td>
<td>35.7</td>
<td>48.9</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>Echolucent</td>
<td>21.4</td>
<td>25.5</td>
<td>35.0</td>
<td>0.31</td>
</tr>
<tr>
<td>Mixed</td>
<td>42.9</td>
<td>25.5</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>Vascular mass (cm²)</td>
<td>0.14±0.04</td>
<td>0.15±0.03</td>
<td>0.14±0.04</td>
<td>0.25</td>
</tr>
</tbody>
</table>

3.8.2 Relationship between carotid ultrasound findings and angiographic coronary artery disease

The table 3.14 shows the comparative analysis of carotid ultrasound findings between those with unobstructed coronary arteries, single vessel disease and
multi vessel coronary artery disease using ANOVA. There was a significant
difference between the groups in the max IMT with plaque thickness and
plaque score, in that, those with single vessel and multi vessel disease had

**Figure 3.2:** Box plots of the IMT measurements in the unobstruced
coronaries, stable CAD and ACS groups.
Table 3.14: Comparison of carotid ultrasound findings between unobstruced coronaries, one-vessel disease and multi-vessel disease

<table>
<thead>
<tr>
<th></th>
<th>No-CAD (N= 31)</th>
<th>1 VD (N= 68)</th>
<th>MVD (N= 46)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min IMT (mm)</td>
<td>0.50± 0.13</td>
<td>0.51± 0.12</td>
<td>0.52± 0.12</td>
<td>0.73</td>
</tr>
<tr>
<td>Mean IMT (mm)</td>
<td>0.64± 0.16</td>
<td>0.67± 0.14</td>
<td>0.69± 0.14</td>
<td>0.42</td>
</tr>
<tr>
<td>Max IMT (mm)</td>
<td>0.80± 0.18</td>
<td>0.83± 0.17</td>
<td>0.85± 0.16</td>
<td>0.42</td>
</tr>
<tr>
<td>IMT-max (mm)</td>
<td>1.24± 0.58</td>
<td>2.00± 1.17</td>
<td>2.00± 1.00</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Plaque Score (median)</td>
<td>0.0</td>
<td>2.0</td>
<td>2.0</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>Plaque Echogenicity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echodense</td>
<td>35.7</td>
<td>41.2</td>
<td>40.0</td>
<td>0.87</td>
</tr>
<tr>
<td>Echolucent</td>
<td>21.4</td>
<td>31.4</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>42.9</td>
<td>27.5</td>
<td>31.4</td>
<td></td>
</tr>
<tr>
<td>Vascular Mass (cm²)</td>
<td>0.14± 0.04</td>
<td>0.14± 0.04</td>
<td>0.15± 0.04</td>
<td>0.28</td>
</tr>
</tbody>
</table>

much higher max IMT with plaque thickness and a higher plaque score. However, when single vessel and multi vessel CAD were compared with each other, there was no significant difference between these two groups in IMT-max and plaque score. The min IMT, mean IMT, max IMT, plaque echogenicity and vascular mass were all comparable between the groups.
Figure 3.3: Receiver-operating characteristic (ROC) curves for the IMT values and plaque score to predict presence of CAD.

Figure 3.3 shows the ROC curve analysis of IMT values for the prediction of CAD. The areas under the ROC curves as shown in the table 3.15, for mean IMT, IMT-max and plaque score were 0.588 (95% CI: 0.471-0.705; P = 0.13), 0.724 (95% CI: 0.635-0.812; P < 0.001) and 0.701 (95% CI: 0.599-0.803);

**Table 3.15:** ROC curve analysis for IMT values and plaque score to predict the presence of CAD

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean IMT</td>
<td>0.59</td>
<td>0.47-0.71</td>
<td>0.13</td>
</tr>
<tr>
<td>IMT-max</td>
<td>0.72</td>
<td>0.64-0.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plaque Score</td>
<td>0.70</td>
<td>0.60-0.80</td>
<td>0.001</td>
</tr>
</tbody>
</table>

AUC= area under the curve, CI= confidence interval
P=0.001) respectively. Spearman’s rank correlation analysis as shown in table 3.16 had shown good correlation of IMT-max and plaque score (r=.317, p < 0.001 and r=.419, p < 0.001) with the presence of CAD.

**Table 3.16: Spearman’s rank coefficient analysis of carotid markers for presence of CAD.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation Coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min IMT</td>
<td>.059</td>
<td>0.48</td>
</tr>
<tr>
<td>Mean IMT</td>
<td>.120</td>
<td>0.15</td>
</tr>
<tr>
<td>Maximum IMT</td>
<td>.122</td>
<td>0.14</td>
</tr>
<tr>
<td>IMT-max</td>
<td>.317</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plaque Score</td>
<td>.419</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vascular mass</td>
<td>.121</td>
<td>0.15</td>
</tr>
</tbody>
</table>

The correlation between the carotid ultrasound markers and severity of CAD based on syntax score as shown in table 3.17 showed that IMT-max was the only marker to predict the severity of CAD (r=.222, p= 0.008).

### 3.9 Comparison of Echocardiographic findings

#### 3.9.1 Comparison of Echocardiographic findings in No-CAD vs. Stable CAD vs. ACS groups

The comparative analysis of the echocardiographic findings between the three study groups, namely, those with unobstruced coronaries (No-CAD), stable coronary artery disease and acute coronary syndrome was performed using
one-way analysis of variance (ANOVA) and the results are shown in table 3.18. There was no significant difference in left ventricular mass index (LVMI).

Table 3.17: Spearman's rank coefficient analysis of carotid markers for severity of CAD based on syntax score

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation Coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min IMT</td>
<td>.103</td>
<td>0.22</td>
</tr>
<tr>
<td>Mean IMT</td>
<td>.109</td>
<td>0.20</td>
</tr>
<tr>
<td>Maximum IMT</td>
<td>.090</td>
<td>0.29</td>
</tr>
<tr>
<td>IMT-max</td>
<td>.222</td>
<td>0.008</td>
</tr>
<tr>
<td>Plaque Score</td>
<td>.162</td>
<td>0.06</td>
</tr>
<tr>
<td>Vascular mass</td>
<td>.074</td>
<td>0.38</td>
</tr>
</tbody>
</table>

or relative wall thickness (RWT) between the groups. There was also no significant difference between the groups in the left ventricular geometric patterns, although there was a trend towards more concentric remodelling and hypertrophy in those with unobstructed coronaries. The systolic function assessed by left ventricular ejection fraction was significantly different between the groups in that the ACS group had lower mean ejection fraction values compared to the other two groups. Similar findings were seen with global circumferential strain. The diastolic function assessed by trans-mitral pulsed wave Doppler showed no significant difference in the E/A ratio between the groups, whereas with tissue Doppler assessment, the E/e’ averaged from the 4 LV walls, namely, septal, lateral, anterior and lateral walls was significantly higher in the stable CAD and ACS groups. The E/e’
values were not different between the stable CAD and ACS groups. Grading of diastolic function based on trans-mitral pulsed wave Doppler and tissue

**Table 3.18: Comparative analysis of the echocardiographic findings in No-CAD, stable CAD and ACS groups.**

<table>
<thead>
<tr>
<th></th>
<th>No-CAD (n=31)</th>
<th>Stable CAD (n=62)</th>
<th>ACS (n=53)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV Mass Index (g/m²)</td>
<td>87.6±23.7</td>
<td>89.7±26.9</td>
<td>92.0±19.5</td>
<td>0.72</td>
</tr>
<tr>
<td>RWT</td>
<td>0.44±0.10</td>
<td>0.42±0.09</td>
<td>0.41±0.08</td>
<td>0.49</td>
</tr>
<tr>
<td>LV Geometry (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>38.7</td>
<td>54.8</td>
<td>52.8</td>
<td>0.53</td>
</tr>
<tr>
<td>Conc remodelling</td>
<td>35.5</td>
<td>29.0</td>
<td>28.3</td>
<td></td>
</tr>
<tr>
<td>Conc Hypertrophy</td>
<td>12.9</td>
<td>8.1</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Ecc Hypertrophy</td>
<td>9.7</td>
<td>8.1</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>68.6±7.4</td>
<td>66.9±8.4</td>
<td>62.3±8.7</td>
<td>0.002</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>0.7±0.3</td>
<td>0.7±0.2</td>
<td>0.7±0.2</td>
<td>0.70</td>
</tr>
<tr>
<td>Average Sm (cm/s)</td>
<td>9.0±1.9</td>
<td>8.2±1.6</td>
<td>7.9±1.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Average e’ (cm/s)</td>
<td>10.1±2.8</td>
<td>8.9±2.4</td>
<td>8.2±2.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Average E/e’</td>
<td>7.9±1.8</td>
<td>9.3±3.1</td>
<td>9.4±2.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Diastolic Function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grading (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>56.7</td>
<td>51.6</td>
<td>43.4</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>36.7</td>
<td>43.5</td>
<td>49.1</td>
<td>0.78</td>
</tr>
<tr>
<td>Moderate</td>
<td>6.6</td>
<td>4.8</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Myocardial Performance Index</td>
<td>0.40±0.16</td>
<td>0.37±0.16</td>
<td>0.40±0.19</td>
<td>0.50</td>
</tr>
<tr>
<td>LA volume (ml)</td>
<td>52.1±16.6</td>
<td>46.6±15.0</td>
<td>41.1±11.4</td>
<td>0.003</td>
</tr>
<tr>
<td>LA volume index (ml/m²)</td>
<td>24.3±9.1</td>
<td>22.6±8.1</td>
<td>20.7±5.1</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Doppler imaging did not show significant difference in diastolic dysfunction between the groups. The systolic (Sm) and early diastolic (e') myocardial velocities averaged from the 4 LV walls, namely, septal, lateral, anterior and lateral walls were significantly lower in the stable CAD and ACS groups. However, there was no difference between the stable CAD and ACS groups in both the parameters. The myocardial performance index was not significantly different between the groups. The LA volume was highest in those with unobstructed coronaries and lowest in the ACS group, but when indexed to body surface area, there was no significant difference between the groups.

3.9.2 Relationship between left ventricular geometry and angiographic coronary artery disease

A comparative analysis was performed between those with normal LV geometry and abnormal LV geometry using student t-test. 74/145 (51%) had normal LV geometry and 71/145 (49%) had abnormal LV geometry, i.e. concentric remodelling, concentric hypertrophy or eccentric hypertrophy. The findings are summarized in the table 3.19. Those with abnormal geometry were significantly older and proportionately more women in the abnormal LV geometry group than in the normal group (25% vs. 11%, p=0.03). Other baseline demographic characteristics including hypertension, diabetes, hyperlipidaemia, smoking were similar in both groups.

There was no significant difference between the groups either in the presence or the clinical presentation of CAD. Also, there was no significant difference in
the severity of CAD between the two groups either by the number of vessels involved or by the more quantitative analysis using syntax score.

The comparison of carotid ultrasound parameters between the two groups showed some interesting findings. The minimum, mean and maximal IMT were all significantly greater in those with abnormal LV geometry. The area under ROC curves as shown in figure 3.4 and table 3.20 for min IMT, mean IMT and max IMT to predict abnormal geometry were 0.63 (95% CI: 0.54-0.72, p = 0.006), 0.62 (95% CI: 0.53-0.71, p = 0.01) and 0.61 (95% CI: 0.51-0.70, p = 0.03), respectively. However, the IMT-max and plaque score were not significantly different between the groups. The difference in vascular mass between the two groups was of borderline significance.

The LV ejection fraction was not significantly different between those with normal and abnormal geometry. As expected, the LV mass index was significantly higher in the abnormal LV geometry group. The trans mitral E/A ratio and septal E/e' were not significantly different, but lateral E/e' was higher in the abnormal geometry group which was reaching statistical significance. There was a trend towards more diastolic dysfunction in the abnormal LV geometry group, but there was no difference in the myocardial performance index. The LA volume index was significantly higher in the abnormal geometry group.
Table 3.19: Comparative analysis of echocardiographic, carotid ultrasound and biomarkers between normal and abnormal LV geometry.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal (n= 74)</th>
<th>Abnormal (n= 71)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>55.1± 10.1</td>
<td>58.9± 10.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>89.2</td>
<td>74.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>40.5</td>
<td>52.1</td>
<td>0.19</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>20.3</td>
<td>31.0</td>
<td>0.18</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>44.6</td>
<td>33.8</td>
<td>0.25</td>
</tr>
<tr>
<td>Hyperlipidaemia (%)</td>
<td>71.6</td>
<td>71.8</td>
<td>1.00</td>
</tr>
<tr>
<td>Waist circumference (%)</td>
<td>99.7± 10.9</td>
<td>101.9± 9.9</td>
<td>0.24</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6± 4.1</td>
<td>28.5± 4.6</td>
<td>0.22</td>
</tr>
<tr>
<td>Presence of CAD (%)</td>
<td>83.8</td>
<td>74.6</td>
<td>0.22</td>
</tr>
<tr>
<td>CAD category (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>16</td>
<td>25</td>
<td>0.41</td>
</tr>
<tr>
<td>Stable CAD</td>
<td>46</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>ACS</td>
<td>38</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Multi vessel CAD (%)</td>
<td>31.1</td>
<td>32.9</td>
<td>0.57</td>
</tr>
<tr>
<td>Syntax score*</td>
<td>8 (0 - 13)</td>
<td>6 (0 -12)</td>
<td>0.45</td>
</tr>
<tr>
<td>Min IMT (mm)</td>
<td>0.49± 0.12</td>
<td>0.54± 0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean IMT (mm)</td>
<td>0.65± 0.13</td>
<td>0.70± 0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>Max IMT (mm)</td>
<td>0.80± 0.16</td>
<td>0.86± 0.17</td>
<td>0.04</td>
</tr>
<tr>
<td>Max IMT with plaque (mm)</td>
<td>1.76± 1.02</td>
<td>1.91± 1.10</td>
<td>0.39</td>
</tr>
<tr>
<td>Plaque Presence (%)</td>
<td>67.6</td>
<td>71.8</td>
<td>0.59</td>
</tr>
<tr>
<td>Plaque score (median)</td>
<td>1.0</td>
<td>2.0</td>
<td>0.22</td>
</tr>
<tr>
<td>Vascular Mass (cm²)</td>
<td>0.14± 0.04</td>
<td>0.15± 0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>65.7± 8.4</td>
<td>65.3± 9.0</td>
<td>0.81</td>
</tr>
<tr>
<td>LV Mass Index (g/m²)</td>
<td>82.6± 15.6</td>
<td>98.3 ± 28.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>0.7± 0.2</td>
<td>0.7± 0.3</td>
<td>0.42</td>
</tr>
<tr>
<td>Dec Time (m/sec)</td>
<td>205.0± 37.1</td>
<td>209.4± 46.9</td>
<td>0.54</td>
</tr>
<tr>
<td>IVRT (m/sec)</td>
<td>93.8± 14.9</td>
<td>93.7 ± 16.2</td>
<td>0.99</td>
</tr>
</tbody>
</table>
### Table 3.20: Receiver-operating characteristic analysis of carotid IMT and plaque score in relation to LV geometry.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min IMT</td>
<td>0.63</td>
<td>0.54- 0.72</td>
<td>0.006</td>
</tr>
<tr>
<td>Mean IMT</td>
<td>0.62</td>
<td>0.53- 0.71</td>
<td>0.01</td>
</tr>
<tr>
<td>Max IMT</td>
<td>0.61</td>
<td>0.51- 0.70</td>
<td>0.03</td>
</tr>
<tr>
<td>IMT-max</td>
<td>0.54</td>
<td>0.45- 0.64</td>
<td>0.41</td>
</tr>
<tr>
<td>Plaque score</td>
<td>0.55</td>
<td>0.46- 0.65</td>
<td>0.27</td>
</tr>
</tbody>
</table>

AUC= Area under the curve, CI= confidence interval.
Figure 3.4: Receiver-operating characteristic curves of carotid IMT and plaque score in relation to LV geometry.

3.10 Comparison of Lipid profile and biomarkers in Unobstructed coronaries vs. Stable CAD vs. ACS

Comparison of lipid profile and biomarkers between the three groups of study participants was done using ANOVA, as shown in table 3.21. There was a significant difference between the groups in the distribution of total and LDL cholesterol levels, in that, the total cholesterol levels were higher in the normal and ACS groups and LDL-C was significantly higher in the ACS group. There was no significant difference in the triglyceride and HDL cholesterol levels between the groups. Apo A1 levels were significantly lower in the ACS group. Hs-CRP levels were significantly higher in the ACS group. There was no significant difference in the fibrinogen and PON1 levels between the groups. Osteopontin levels were significantly higher in the ACS group of patients.
Table 3.21: Comparison of serum biomarkers in the three groups

<table>
<thead>
<tr>
<th></th>
<th>No-CAD (n=31)</th>
<th>Stable CAD (n=62)</th>
<th>ACS (n=53)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.8 ± 1.1</td>
<td>4.0 ± 1.2</td>
<td>5.0 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>2.2 ± 1.1</td>
<td>1.9 ± 1.2</td>
<td>1.6 ± 1.0</td>
<td>0.08</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>0.28</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2.5 ± 1.1</td>
<td>2.1 ± 0.9</td>
<td>3.1 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/l)*</td>
<td>2.1</td>
<td>1.9</td>
<td>12.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.8± 1.0</td>
<td>2.9± 0.7</td>
<td>3.0± 0.7</td>
<td>0.19</td>
</tr>
<tr>
<td>Apo A1 (g/l)</td>
<td>1.7± 0.3</td>
<td>1.7± 0.3</td>
<td>1.5± 0.3</td>
<td>0.000</td>
</tr>
<tr>
<td>PON 1 (nmol/ml/min)</td>
<td>103.3± 69.5</td>
<td>88.5± 59.7</td>
<td>83.1± 52.4</td>
<td>0.56</td>
</tr>
<tr>
<td>Osteopontin (ng/ml)</td>
<td>63.9± 42.7</td>
<td>59.3± 46.6</td>
<td>97.9± 69.6</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Median
CHAPTER 4
Chapter 4: Discussion

4.1 Demographic characteristics

The demographic profile of the subjects in this study shows some interesting findings. The mean age of the study population was comparable to most of the studies done in similar populations. The male to female ratio was about 4:1. It is well known that women are at significantly less risk of developing CAD compared to men and studies have consistently found male preponderance in the prevalence of CAD and also at least two-fold higher mortality rates related to CAD (182). The ethnic diversity (20% ethnic minority groups) of the study population reflects the local population. The data published by the National Office of Statistics in 2009 estimated that ethnic minority groups constitute about 23% of the Greater Manchester population.

The traditional cardiovascular risk factor profile in the study population shows that the majority of patients had at least two risk factors and a quarter of the group had diabetes. A very high proportion of the subjects were already on secondary preventive medications including aspirin, beta-blockers and statins. More than 70% of the study population were either overweight or obese and this is comparable with the recent data published from United States where it is estimated that more than 60% of the adult population were either overweight or obese (183).

The demographic comparison between the three groups of subjects recruited in this study, namely, those with unobstruced coronaries, stable coronary artery disease and acute coronary syndrome (ACS) showed some surprising results. Perhaps not unexpectedly, those with unobstructed coronary arteries
were younger than those with angiographically significant coronary artery disease, but such a high prevalence of risk factors in the former group was surprising. However, these subjects were not healthy controls and were referred for coronary angiography due to the fact that they had symptoms, risk factors and most of them (81%) had some form of positive stress test (treadmill exercise, dobutamine stress echocardiography or myocardial perfusion scan) before undergoing invasive coronary angiography.

Another interesting finding was the high prevalence of abnormal glucose levels in the study population with 20% and 29% in the IFG and diabetic range respectively. The association of diabetes and cardiovascular disease is well known, but in the recent years there has been more focus on pre diabetic state (either impaired fasting glucose or impaired glucose tolerance). A recent systematic review of 18 large studies consisting of over 175,000 participants found that IFG is associated with a risk ratio of 1.20 (95% CI: 1.12-1.28) for cardiovascular disease (184). In our study, 21% of the patients in the ACS group who did not have history of diabetes had IFG. However, it is not clear as to what extent stress related hyperglycaemia secondary to the acute coronary event will have contributed to the raised glucose levels.

In both stable CAD and ACS groups about 30% of subjects had multi-vessel disease. In previous studies comparing carotid ultrasound markers and angiographic coronary artery disease, the prevalence of multi-vessel disease has varied from 10-60% (66, 185-188).
4.2 Carotid Ultrasound markers and coronary artery disease

4.2.1 CIMT in predicting the presence and severity of CAD

In our study, there was no correlation between IMT values and coronary artery disease. We adhered strictly to the protocol as recommended by the consensus statement in that IMT was measured at far wall of CCA in a segment, which was plaque free. A number of previous CIMT studies, which found correlation with manifest CAD, had pitfalls in the methodology of IMT measurement. The main limitation in such studies was that there was no clear distinction between IMT and plaque and the reported IMT values may have incorporated plaque thickness measurements. This has been mentioned in a recent meta-analysis of population based and diagnostic cohort studies done by Inaba et al where they found 27 out of 35 studies (77%) included, did not clearly state whether plaque was included in their IMT measurements (110). In the diagnostic cohort studies arm of this meta-analysis, CIMT, compared to carotid plaque was found to be less predictive of obstructive CAD. There has been considerable heterogeneity amongst the studies in arterial segments used to analyse CIMT. It is well known that the IMT measurements taken from the carotid bulb and ICA segments are less reliable due to their anatomical location and also are more likely to include plaques due to the non laminar flow patterns in these segments. In the sub-group analysis of the meta-analysis done by Inaba et al, CCA-IMT, compared to CB and ICA IMT measurements correlated less with angiographic CAD (110). Lorenz et al in their meta-analysis called for a standardized protocol in future studies due to the significant heterogeneity amongst studies in the ultrasound protocol for measurement of IMT (75). In the study by Adams et al, where CIMT was
measured in plaque free regions in the CCA, mean IMT values only weakly correlated with angiographic coronary artery disease and there was no value of IMT in the ROC curve analysis that had a sensitivity and specificity of >80% simultaneously to predict CAD (188). When we recalculated the IMT values by incorporating the maximal plaque thickness in to the maximal IMT values, this was significantly higher in those with obstructive CAD we found it significantly correlating with presence of CAD (r= .317, p <0.001). A recent study from the ARIC data reported that including plaque information onto CCA-IMT values improved prediction of CHD risk (189).

The comparative analysis of carotid ultrasound findings between the three groups of study participants based on severity of coronary artery disease, namely, those with unobstructed coronary arteries, single vessel disease and multi vessel disease has shown some interesting findings. We found no difference in IMT values between the groups, irrespective of the severity of CAD. This is in contradiction to the findings from some previous studies, which found that IMT values were predictive of severity of CAD (27, 66). Kablak-Ziembicka et al and Crouse et al reported the average of maximal IMT thicknesses from multiple segments as mean IMT and we now know that this included plaques in the IMT measurement. Also, there was considerable overlap in the distribution of IMT values between the groups based on number of vessels involved and the authors were unable to provide cut off values that could be predictive of severity of CAD. In another cross sectional study, Sakaguchi et al found that IMT and plaque score were equivalent in predicting severity of CAD. However, they found CCA-IMT to be least predictive of the
markers, compared to bulb and ICA-IMT values, and this was despite including plaque measurements in the IMT values (107). Our study finding is supported by a previous study by Morito et al, in which CIMT was not found to be an independent predictor of severity of CAD and in this study, the mean IMT was reported as averaged IMT from both distal CCAs (185). Also, the REACH registry follow-up data of 2,317 subjects with pre-existing atherosclerotic vascular disease showed that CIMT was not associated with increased cardiovascular morbidity or mortality (190).

When plaque thickness was incorporated in the IMT measurement to derive IMT-max, this correlated with severity of CAD assessed by syntax score ($r= .22$, $p= 0.008$). Similarly, in our study, IMT-max score correlates with both the presence and severity of CAD. However, previous studies, which reported IMT to correlate with coronary artery disease have either been not clear if they had included plaque measurements in IMT or they had included plaque thicknesses in IMT values. Inaba et al have highlighted this in a recent meta-analysis, in that, 77% of studies were not clear about the methodology of IMT measurement (110). In a recent study by Ikeda et al, mean IMT and plaque score were found to be predictive of the presence and severity of CAD assessed by syntax score. However, in this study, the mean IMT reported was the average of the maximal IMT thickness in the CCA and the authors report incorporating the plaque thickness in to the mean IMT values. Hence this value is similar to the IMT-max value we report in out study (191).
4.2.2 Carotid plaque in predicting presence and severity of CAD

In our study, carotid plaque was found in a significantly higher proportion of subjects with manifest CAD compared to those with unobstructed coronary arteries by angiography. This has been shown in previous similar studies (185, 192, 193). As expected, the carotid plaque score was significantly different between those with unobstructed coronary arteries and those with obstructive CAD. Also, plaque score significantly correlated with presence of CAD (r= .419, p <0.001). This has been shown in previous similar studies (185, 192, 193). However, there was no significant difference in the plaque scores between those with single vessel and multi vessel disease and plaque score did not correlate with severity of CAD assessed by syntax score (r= .162, p= 0.06). Ikeda et al, as mentioned above found the plaque score to be predictive of presence and severity of CAD assessed by syntax score (191). However, the study was done in a more selective population, only 54% of participants were investigated for ischaemic heart disease and also had not included patients with ACS. Moreover, the definition of plaque used in their study was different to our study and did not accord to the recommendations of the Mannheim Consensus statement. Morito et al, in their study, found that the plaque score was an independent predictor of severity of CAD (OR 1.33; 95% CI 1.20- 1.48) (185). However, difference in baseline characteristics between those with and without CAD in that study was much greater than in our study and also the study population was much older than in our study. In another study, diabetic subjects with no previous known CAD underwent carotid ultrasonography and multi-slice CT coronary angiography and they found that carotid plaque score correlated with the presence and extent of
CAD (194). However, this study was done in a highly selective diabetic population and also did not compare carotid ultrasound with invasive coronary angiography, which is considered to be the gold-standard test for identifying obstructive CAD.

4.2.3 Carotid plaque in predicting ACS
There was no significant difference in the carotid plaque scores between those with stable CAD and ACS. This was similar to the finding from the study by Seo et al (98). Carotid plaque morphology was not significantly different between the groups although there was a trend towards more echodense plaque in those with stable coronary disease. It has been noticed that the ACS group patients are more likely to have more echolucent and complex plaques in previous studies (98, 187, 195). We did not find similar observation in our study, but our finding is supported by a recent study, which compared carotid plaque morphology between stable CAD and ACS patients using high-resolution magnetic resonance imaging, and found that there was no significant difference in the carotid arterial morphology between the groups (196).

4.2.4 Vascular mass and CAD
Vascular mass of the carotid arteries was not significantly different for those with and without obstructive CAD. In a population study, it was found that IMT-cross sectional area (vascular mass) was correlated with a higher risk population, but no hard end points were determined for clinical outcome in this study (197). There are no previous diagnostic cohort studies to our
knowledge, which looked at carotid vascular mass in relation to angiographic coronary artery disease. It is possible that, carotid vascular mass alterations are likely a remodelling process secondary to hypertension and aging and not a reflection of the true atherosclerotic process.

4.3 Left ventricular geometry and function in relation to carotid atherosclerosis and coronary artery disease

In our study, the presence of carotid plaque and plaque score were not significantly different between those with normal and abnormal LV geometry. However, we found that the carotid IMT scores (min IMT, mean IMT and max IMT) were significantly higher in those with abnormal LV geometry. In a recent cross-sectional study by Ciccone et al, CIMT assessed by a radiofrequency technique was found to have correlation with LVMI and severity of CAD (198). However, this study was limited by the fact that, there was no control group with unobstructed coronary arteries for comparative analysis. Furthermore, the severity of CAD was assessed qualitatively, whereas, in our study we have performed a more robust quantitative analysis in the form of syntax scoring to assess the severity of CAD in addition to the qualitative analysis. Another population-based study, the Cardiovascular Health Study found correlation between LV mass and CIMT (r= .20, p < 0.01), but the relationship with abnormal geometry and CAD was not studied (199). We found the carotid vascular mass to be higher in those with abnormal LV geometry. Roman et al demonstrated a similar finding in their study comparing hypertensive with normotensive subjects, and found carotid vascular area to be correlated with LV mass although neither the LV geometric patterns nor
their relation with CAD were assessed (200). Overall, the carotid ultrasound findings in our study highlight that IMT and plaque may be determined by different pathological processes; IMT is more likely to be related to a remodelling phenomenon with trophic changes of vascular smooth muscle rather than a reflection of the true atherosclerotic process.

In our cross-sectional study, there was no association between abnormal geometry and clinically manifest coronary artery disease. In previous longitudinal studies, adverse LV remodelling in the form of concentric and eccentric hypertrophy and concentric remodelling were found to predict cardiovascular events and were associated with worse prognosis in hypertensive and CAD patients (167, 168, 201). However, to our knowledge there is no study, which has assessed the relationship between left ventricular geometry and angiographic coronary artery disease. One study looked at left ventricular geometric patterns and coronary plaque burden by cardiac CT in patients without LV hypertrophy and found that increased LV mass and concentric remodelling correlated with coronary plaque burden (202). Surprisingly, we found a non-significant trend towards more abnormal LV geometry in the group with unobstructed coronaries and no difference in the proportion of hypertensive subjects between the three groups. As mentioned earlier, those with unobstructed coronary arteries had significant cardiovascular risk factors and positive stress tests. It is possible that the falsely positive stress tests were in part a reflection of the abnormal LV geometry and reduced coronary flow reserve in many of these patients.
As expected, the LVEF was significantly lower in the ACS group compared to the other two groups, as these patients had by definition incurred a degree of myocardial damage. Myocardial systolic velocity measurements by tissue doppler imaging were similar in the groups. This finding highlights the importance of assessing changes in myocardial thickening rather than velocity as the latter may be affected by pushing and pulling forces and represents more crude changes in wall motion when measured from a single point within the myocardium. Nevertheless, myocardial systolic and early diastolic velocities were able to differentiate those with unobstructed coronary arteries from those with manifest coronary artery disease. Similar findings were seen in a recent retrospective registry data, where the peak systolic and early diastolic velocities were significantly lower in subjects with CAD compared to the control group and was an independent predictor of CAD in patients who were investigated for suspected stable angina pectoris, OR: 1.7 (95% CI: 1.1–2.5, P < 0.01) and OR: 1.4 (95% CI: 1.1 – 1.8, P, 0.01), respectively (203). It is possible that this finding reflects ischaemia in the subendocardial layer, where the longitudinal myocardial fibres are located, leading to early diastolic and longitudinal systolic dysfunction.

The averaged E/e’ from the 4 LV walls was higher in those with obstructive CAD, but not significantly different between stable CAD and ACS groups. The study mentioned above by Hoffman et al, also found that the averaged E/e’ values were significantly lower in those with obstructive CAD, but in a multivariable model, it was not found to be predictive of CAD (203). However, that study had only a selected population, namely, those with stable CAD,
whereas, in our study, we had a wide spectrum of patients with unobstructed coronaries, stable CAD and ACS. The ACS patients in our group did not have previous history of myocardial infarction and hence it is likely that the changes we found represent the left ventricular function prior to the event.

LA volume was higher in the normal group compared to those with obstructive CAD. When the LA volume was indexed to body surface area (LAVI), there was a non-significant trend towards higher volume in the normal group. In a previous study on patients presenting with chest pain, LA volume and LAVI done using cardiac CT were found to be higher in the ‘risk factor’ and ACS groups, compared to healthy control group. However, LAVI was not found to be predictive of ACS in the multivariable model (204). Our study is limited by not having a healthy control group.

Myocardial performance index (MPI) has not been found useful in our study to differentiate those with significant CAD from angiographically unobstructed coronary arteries. Previous studies done in post MI patients did not find MPI to be a useful marker to predict adverse outcomes (205-207).

There was no significant difference in systolic function between the 2 groups based on LV geometry and there was a non-significant trend towards more diastolic dysfunction in the abnormal geometry group. Several studies have reported correlation between left ventricular hypertrophy and diastolic dysfunction in hypertensive subjects (200, 208, 209), but data on the relation between different LV geometrical patterns and diastolic function in relation to
CAD is very limited. In a previous study, abnormal LV geometry was found to be associated with diastolic dysfunction after adjusting for age and hypertension (210). However, one-third of subjects in that study had LV systolic dysfunction and this might have influenced the results. In our study there was no significant difference in the prevalence of hypertension between the groups and only 10% of the study subjects had LV systolic dysfunction. Andren et al, in their study of elderly subjects found more association between abnormal LV geometry and diastolic dysfunction in those with coronary artery disease. The association was heterogeneous in the degree of dysfunction with different forms of abnormal LV geometry in that, those with hypertrophic patterns (concentric and eccentric) had more left atrial enlargement, more prolonged IVRT with concentric hypertrophy, prolonged deceleration time with eccentric hypertrophy and a restrictive filling pattern (E/A ration >2) in the concentric remodelling pattern (211). However, aging per se influences diastolic function and the extent to which CAD had an effect on diastolic dysfunction is not known. In our study, we found that the left atrial volume index (LAVI) was significantly greater in those with abnormal LV geometry as shown in the study by Andren et al. A similar finding was shown in a very large clinical retrospective cohort of over 36,000 patients with preserved systolic function and in addition they found that increased LAVI was an independent predictor of mortality (212).
4.4 Biomarkers

4.4.1. Biomarkers and coronary artery disease

Comparative analysis of the lipid profile showed that the ACS group of patients had the worst profile, in that, they had the highest total and LDL cholesterol levels amongst the 3 groups. From large-scale epidemiological studies (e.g. Framingham Heart Study), it has been strongly established that raised total cholesterol and LDL-C levels are associated with increased cardiovascular events and worse outcomes in subjects without previous CAD and after myocardial infarction (157, 213-219). Furthermore, effective lipid modification strategies, (e.g. statins) have also shown to improve the cardiovascular outcomes in many interventional studies (220-222). Rather surprisingly, we found that those with stable CAD had better values compared to those with unobstructed coronary arteries. However, a very high proportion of these patients had history of hyperlipidaemia (87%), highest amongst the three groups and most of them were already established on lipid modifying treatment. There was no significant difference in the HDL-C levels between the groups, but Apo A1 levels were significantly lower in the ACS group. A previous study had identified Apo A1 levels to be lower in acute myocardial infarction patients after adjusting for baseline characteristics and other lipid levels (156).

The hs-CRP levels were significantly elevated in the ACS group, but not different between normal and stable CAD groups. The meta-analysis by Danesh et al of population-based studies had shown raised CRP levels to be associated with increased risk of major coronary events (129). There was no
difference in fibrinogen levels between the 3 groups of patients. In a previous study, fibrinogen was found not to have predictive value for cardiovascular events when compared to CRP (142). In our study, there was no significant difference in the paraoxonase 1 (PON1) activity levels between the groups. In a previous follow-up study, baseline PON1 levels were found to be 20% lower in those who had a coronary event. However, the PON1 concentration was not significantly different between those who did and did not have a coronary event (152). This study is limited by including only men in the follow-up. We found that the osteopontin levels were significantly higher in the ACS patients. A similar finding was seen in a previous study, where osteopontin levels were raised in subjects with NSTEMI compared to stable CAD patients. However, it did not correlate with severity of disease (149).

4.4.2 Biomarkers and LV geometry

There were no significant differences between the 2 groups (normal and abnormal LV geometry) in the biomarkers measured (hs-CRP, fibrinogen, osteopontin, paraoxonase 1 and Apo A1). A recent study found significantly higher values of hs-CRP in those with abnormal LV geometry (223). However, this was in a selective, elderly population and their cardiovascular profile and the presence of CAD were not accounted for in the analysis. In another study involving the Framingham offspring follow-up study, participants underwent various biomarker analysis and echocardiographic assessment of LV geometry. CRP and fibrinogen levels were elevated in those with abnormal LV geometry, although in multivariable analysis these markers were not statistically significant (224). Osteopontin levels were found to be raised in
those with LVH and diastolic heart failure in a recent study, but osteopontin was not predictive of LVH or diastolic heart failure in a multivariable model (225). To the best of our knowledge, there are no reported studies of Apo A1 and paraoxonase 1 in relation to LV geometry.
CHAPTER 5
Chapter 5. Summary and conclusion

5.1 Carotid plaque and severity of CAD
In our study, there was association between carotid plaque score and obstructive CAD. However, we found no correlation between the extent of carotid plaque and severity of coronary artery disease. This finding is likely due to the fact that the extent of atherosclerosis is quite varying amongst different vascular beds due to local hemorheologic influences. We did not find significant difference in the plaque morphological pattern between stable and acute coronary syndromes although there was a trend towards more echolucent plaque in the ACS patients.

5.2 Carotid ultrasound markers in predicting CAD
There was no correlation between CIMT and clinically manifest coronary artery disease in our study. We found carotid plaque to be a better predictor of obstructive coronary artery disease. Also, interestingly, we have shown that a composite score consisting of IMT and plaque (IMT-max) to be associated with CAD. Carotid plaque and CIMT are likely to represent different pathological process. Thickening of the arterial wall without plaque formation might be representation of a remodelling process, whereas carotid plaque is a reflection of the true atherosclerotic process. From this study, it appears that identifying carotid plaque might be preferable to CIMT measurement in risk stratifying subjects presenting with chest pain and to predict angiographic coronary artery disease.
5.3 Left ventricular geometry and function in carotid atherosclerosis and CAD

CIMT correlated with left ventricular mass and abnormal LV geometry, whereas carotid plaque did not correlate with abnormal LV geometry. There was no association between abnormal LV geometry and obstructive CAD in our study. Moreover, there was a trend towards more abnormal LV geometry in those with unobstructed coronaries. As mentioned previously, a very high proportion of these subjects had a positive stress test and this to a certain extent might be a reflection of reduced coronary flow reserve in many of these patients. The myocardial systolic and early diastolic velocities and E/e’ ratio were able to differentiate those with unobstructed coronary arteries from those with manifest coronary artery disease. This might be a reflection of early regional and global diastolic function which are not identified by conventional 2-D and pulsed doppler assessments. However, in ACS patients, the assessment of global systolic function seems to be better in assessing the overall LV function in comparison to tissue velocities, suggesting the importance of assessing the changes in myocardial thickening in this group.

5.4 Serum biomarkers in predicting CAD and LV geometry

In our study, the established biomarker, hs-CRP and a novel biomarker in the form of osteopontin were found to be significantly raised in ACS patients. This reflects the inflammatory state associated with acute coronary event. In our study, HDL associated lipoprotein Apo A1 levels were found to be lower in the ACS group and with no significant difference in the HDL-C levels between the groups, it is possible that estimation of Apo A 1 might be a better predictor of
cardiovascular events than HDL-C. We found no correlation between the serum biomarkers and abnormal LV geometry in our study.

5.5 Strengths of the study

We only included ACS patients who were first time presenters and the patients in the stable CAD group had no previous known ACS. Hence the assessments, which we performed, were more robust in terms of characterisation of the carotid arterial system, echocardiographic parameters and serum biomarker assays.

Patients with significant valvular heart disease, cardiomyopathies, cardiac arrhythmias were not included in the study. These conditions are likely to affect the ventricular geometry and remodelling patterns. Also, subjects who had previous carotid or coronary interventional procedures (both percutaneous and surgical) were excluded as these are very likely to alter the natural course of the disease.

We studied the full spectrum of coronary artery disease by including patients with stable angina, unstable angina, NSTEMI and STEMI.

The carotid ultrasound assessment was performed in accordance to the Manheim consensus recommendations thereby reducing the chance of heterogeneity with the imaging protocol.

All our research procedures were performed at a single centre prospectively by a single operator.

Intra and interobserver variability measurements were performed showing good correlation.
5.6 Limitations of the study

There are a number of limitations in our study. The sample size of our study is small and hence the difference between sub-groups (e.g., gender, ethnicity) could not be analysed. We had expected to recruit more subjects into the study, but we were limited by the changes in the circumstances with regards to the referral pattern of patients for diagnostic coronary angiography after the commencement of patient recruitment in our study. The number of elective diagnostic procedures done at the centre had considerably reduced and this was partly due to the fact that the District General Hospitals around Greater Manchester region had started performing the angiographic procedures in their own centre rather than referring them across to our centre and new evidence that had emerged in terms of management of stable CAD patients (e.g. COURAGE trial) might have influenced the physicians on deciding the diagnostic pathway of patients presenting with chest pain.

A significant proportion of ACS patients from peripheral hospitals were admitted for cardiac catheterisation procedure and returned to their respective hospitals on the same day and these patients could not be recruited into the study due to logistic reasons. Moreover, we had stringent inclusion criteria in our study (e.g. first time ACS presentation) and hence a significant proportion of subjects could not be included in our study. Hence our results from the selective population cannot be applied to a general population.

Due to limited numbers in each group, a multivariable analysis could not be performed to correct for traditional risk factors and other possible confounding factors. However, prevalence of most of the cardiac risk factors were not significantly different between the groups.
We did not have an age and gender matched healthy control group. It was somewhat unexpected that the risk profile of those with unobstructed coronaries would be of the order observed in our study. However, one of our objectives was to identify the carotid markers that best correlated with angiographic coronary artery disease and in that respect we had a control group in the form of those with unobstructed coronary arteries. It would have been difficult to prove the absence of significant coronary artery disease without performing an angiogram in apparently healthy individuals and ethically it would be difficult to justify performing an invasive procedure in an asymptomatic healthy subject.

A proportion of subjects, especially the diabetic population were already established on statin and ACE inhibitor therapy and this is likely to have made an impact on inflammatory biomarkers and vascular remodeling. However, the proportion of these subjects were not significantly different between the groups and hence, the measured differences in the markers will not be accounted for, by the above interventions.

Carotid plaque morphology was not assessed by quantitative methods in our study. However different quantitative methods studied have been found to have issues with reproducibility in previous reviews. Also, contrast enhanced carotid ultrasound was not performed in our study for plaque neovascularisation which might have been useful to assess vulnerable plaque.

In our study, left ventricular volume and mass were calculated using echocardiography. The current gold standard for these measurements is
cardiac MRI scan. Alternatively, 3D echocardiography could possibly have been useful, but both of these modalities were not available at our centre during the study period. However, in our study, the intra and inter observer variability of these measurements were well within the acceptable range.

We did not perform quantitative coronary angiography to estimate the severity of coronary stenoses in our study. However, we have performed Syntax scoring, which is a semi quantitative technique and has been proven to be robust for estimating the severity and complexity of coronary lesions.

In our study, coronary CT angiography was not performed to assess the coronary plaque burden and this might have provided a more accurate estimate as compared to invasive coronary angiography. The ACS group of patients in our study will need to have invasive coronary angiography due to the clinical indication and most of the subjects will have received revascularisation procedures during the index hospital admission. This will considerably alter their coronary anatomy for subsequent investigation like CTCA. Moreover, we did not have access to cardiac CT at the time when this study was conducted.

Our study was cross-sectional in design and hence the prognostic value of our findings cannot be assessed.

5.7 Clinical impact of the study

Carotid intima-media thickness is being increasingly used as a surrogate marker of atherosclerosis in population-based studies and as an end-point measure in interventional studies. In our study, we found that, in those with clinically manifest coronary artery disease, CIMT measurements were not
significantly different compared to those with unobstructed coronary arteries. Furthermore, carotid plaque and a composite score of CIMT with plaque correlated with angiographic coronary artery disease and hence identifying carotid plaque by a non-invasive method might help identify high-risk patients with pre-clinical atherosclerosis and to initiate aggressive risk modification strategies. This simple tool could also be integrated onto the diagnostic pathway for risk stratifying patients presenting with chest pain.

5.8 Future work
Longitudinal follow-up data will allow for assessment of progression of the carotid ultrasound findings, echocardiographic markers and cardiovascular outcomes in these subjects. Larger clinical trials in a similar population will help identify prognostically significant markers, especially in sub-group populations like metabolic syndrome and ethnic minority groups.

5.9 Conclusion
In our study, carotid plaque was found in a significantly higher proportion of subjects with manifest CAD compared to those with unobstructed coronary arteries by angiography. However, there was no significant difference in the carotid plaque scores between those with stable CAD and ACS or between those with single vessel and multi vessel disease and plaque score did not correlate with severity of CAD. Carotid plaque and not intima-media thickness correlated with clinically manifest coronary artery disease. Identifying carotid plaque and a composite
score of CIMT with plaque might be useful to risk stratify symptomatic, at risk population.

There was no association between abnormal LV geometry and obstructive CAD in our study. However, we found that the carotid IMT scores were significantly higher in those with abnormal LV geometry. Assessment of myocardial tissue velocities and diastolic function might help identify patients with obstructive CAD along with other conventional methods. Novel biomarkers like osteopontin and Apo A1 are promising and further studies are required to confirm their utility as risk stratifying tools in patients with acute coronary events.
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Publication


Presentation

APPENDICES

Appendix I: Participant information sheet

Appendix II: Patient consent form for participation in the study

Appendix III: Letter informing the GP about participation of subject in the study

Appendix IV: Study source document

Appendix V: Echocardiography and carotid ultrasound analysis sheet

Appendix VI: Publication

Appendix VII: Abstract

Appendix VIII: CIMT accreditation certificate
PARTICIPANT INFORMATION SHEET

Project Title: Carotid atherosclerosis and left ventricular geometry in coronary artery disease

Principal Investigator: Dr R S Khattar

- We would like to invite you to take part in a research study aiming to identify the carotid artery (artery in the neck) measurements that are closely linked to coronary artery (artery of the heart) disease
- This sheet provides you with the information about the study and how it involves you.
- 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 before deciding on whether to take part or not.

What is the purpose of the study?

Studies have shown good relationship between the severity of disease in the carotid arteries (supplying the head and neck) and in the coronary arteries (supplying the heart). Indeed, the majority of patients with carotid atherosclerosis (furring of arteries) may go on to have an acute heart attack rather than a stroke. The extent and severity of carotid artery disease (atherosclerosis) can be readily detected non-invasively by
ultrasound techniques and this may provide insight into the state of the coronary arteries. Moreover, certain carotid artery disease characteristics might be able to identify not just those with angina, but also those most likely to experience a heart attack. In addition, changes in size, shape and function of the left ventricle (the main pumping chamber of the heart) might also influence the chances of developing a heart attack. This study aims to identify the carotid artery measurements that most closely relate to coronary artery disease and to compare carotid artery characteristics and left ventricular geometry in patients with unobstructed coronary arteries, angina and acute coronary disease.

**Who is doing the study?**

This study is being run by the Manchester Heart Centre at the Manchester Royal Infirmary. The principal investigator (Dr. R S Khattar) is a Consultant Cardiologist in this department. This study has been registered with the University of Manchester for obtaining Doctoral qualification (MD) of the research fellow (Dr S Balakrishnan Nair) involved in this study.

**Why have I been chosen?**

You have been chosen for this study either because you have coronary artery disease or because you have risk factors for future cardiac events.

**Do I have to take part?**

It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and you will be booked in for a study appointment at the Manchester Heart Centre. 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. For your safety the study staff will, if you agree this, inform your family doctor about your participation in this study.

**What happens if I take part?**

If you are considered suitable for the study, we will arrange an appointment for you to come to the Manchester Heart Centre in Manchester Royal Infirmary for the study
visit. You will undergo a full history and physical examination including assessment of coronary risk factors and documentation of heart rate, blood pressure and waist circumference. You will then have an ultrasound scan of the heart and the arteries of neck and blood tests for heart disease. If we cannot obtain adequate ultrasound images, you will be withdrawn from the study.

**What are the possible benefits of taking part?**

You will receive additional testing for the heart condition. A full and detailed cardiac and vascular assessment will be performed including the assessment of your individual risk factors for future cardiovascular events.

**What if new information becomes available?**

Sometimes during the course of a research study, new information becomes available. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw, your study doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

**What are the costs of taking part?**

You will not be paid a fee to take part in the study.

**What if there is a problem?**

If you have a concern about any aspect of the study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can contact the Patient Advice and Liaison Service (PALS) office at Manchester Royal Infirmary, Phone number: 0161 2768686 for further details about NHS Complaints Procedure.

In the event that something does go wrong and you are harmed during the research and this is due to someone’s negligence then you may have grounds for a legal action for compensation against Central Manchester and Manchester Children’s NHS Trust,
but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

**Will my taking part in the study kept confidential?**

All the clinical information you provide will be encoded (so that your personal details such as name and address are secure) and stored in a password protected computer securely.

Some parts of your medical records and the data collected for the study will be looked at by representatives of regulatory authorities and by authorised people to check that the study is being carried out correctly.

**What will happen to my samples?**

Your samples will be encoded so that your personal details are not stated on the sample. After analysis your blood samples will be stored for future research pending ethics committee approval.

**What will happen to the results?**

The results will be published in medical journals. However, no individual will be identifiable from these.

You will also be invited to discuss the findings of the study with the principal investigator

**Have the ethics committee approved this study?**

The study has been approved by the Cumbria and Lancashire B Research Ethics committee.

If you wish to obtain further advice about this research you may contact:

**Study Investigators:**

1. **Dr R S Khattar**  
   Consultant Cardiologist  
   Manchester Heart Centre  
   Oxford Road, Manchester  
   M13 9WL  
   Tel: 0161 276 6576

2. **Dr Satheesh B Nair**  
   Clinical Research Fellow  
   Manchester Heart Centre  
   Oxford Road, Manchester  
   M13 9WL  
   Tel: 0161 276 5427
CONSENT FORM FOR STUDY PARTICIPANTS

Title of Project: Carotid atherosclerosis and left ventricular geometry in coronary artery disease
Principal Investigator: R S Khattar

1. I confirm that I have read and understand the information sheet dated 12/02/2009 version 1.2 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. 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.

3. I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from the Trust, from regulatory authorities, where it is relevant to my taking part in this research.

4. i) I am happy to give permission for collection of blood samples to be used for this study
   ii) I also agree for the blood sample to be stored for any future research that may be performed due to the results of this study.
   I also understand that the sample will be disposed of according to the Trust policy if no longer required or usable.

5. I agree to undergo other tests involved as part of the study and for my General Practitioner to be informed about my participation in the study.

6. I agree to take part in the study.

_____________________________  ___________________  ___________________
Name of Participant              Date                  Signature

_____________________________  ___________________  ___________________
Name of Person taking consent   Date                  Signature
Date:

**Title of Project:** Carotid atherosclerosis and left ventricular geometry in coronary artery disease

**Principal Investigator:** R S Khattar

Dear Dr,

Re: Patient name:

DOB:

The above patient has been recruited to a study, which is currently running in the Department of Cardiology, Manchester Royal Infirmary.

The study is looking at the carotid atherosclerotic patterns and left ventricular geometry by ultrasound technique in patients with coronary artery disease.

I have enclosed a copy of the patient information sheet regarding this study.

The study results will be available as a summary report and we could send you a copy in due course.

I thank you for your co-operation in this matter. Please do not hesitate to contact me if you have any concerns or questions.

Yours Sincerely,

Dr R S Khattar
Consultant Cardiologist
Appendix IV

Carotid Atherosclerosis and LV geometry in CAD - Source document

Subject Name:  
Subject ID:  
Gender:  M/ F  
Age:  
Race:  
Ethnicity:  
Date of visit:  
Weight:  
Height:  
BMI:  
Waist circumference:  
BP:  
HR:  

Risk factors

Hypertension  Yes [ ] No [ ]
Diabetes  Yes [ ] No [ ]
Hyperlipidemia  Yes [ ] No [ ]
Smoking  Yes [ ] No [ ]
Family H/O CAD  Yes [ ] No [ ]

Symptoms and Presentation

Angina  Yes [ ] No [ ]
If yes, stable  Yes [ ] No [ ]
ACS  Yes [ ] No [ ]
STEMI  Yes [ ] No [ ]
NSTEMI  Yes [ ] No [ ]
Prior MI  Yes [ ] No [ ]
Previous PCI  Yes [ ] No [ ]
Previous CABG  Yes [ ] No [ ]
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Appendix V

Data Analysis sheet

Patient ID:

Echo analysis

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Carotid Analysis

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Carotid Plaque

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Appendix VI

Publication

Carotid intima—media thickness: ultrasound measurement, prognostic value and role in clinical practice

Satheesh Balakrishnan Nair,1,2 Rayaz Malik,1,2 Rajdeep S Khattar3

ABSTRACT

Ultrasound measurement of carotid intima—media thickness (IMT) has become a valuable tool for detecting and monitoring progression of atherosclerosis and recently published recommendations provide guidance for proper standardization of these measurements. Important determinants of carotid IMT include age, gender, systolic blood pressure, diabetes mellitus and serum cholesterol levels. Many studies have shown carotid IMT to correlate with the severity of coronary atherosclerosis assessed by CT coronary calcium scores, coronary angiography and intravascular ultrasound. Consistent with its correlation with cardiovascular risk factors and coronary artery disease, a meta-analysis of large observational studies has shown carotid IMT to be a strong predictor of future cardiovascular events. Moreover, in patients with established coronary artery disease a reduction in carotid IMT has been shown to translate into a reduction in future cardiovascular events. Consensus statement now also recommend carotid IMT measurements to further refine the prognostic assessment of patients traditionally considered to be at an intermediate risk of cardiovascular disease.4

INTRODUCTION

The atherosclerotic process is a generalised process affecting the arterial tree which begins in childhood, progresses over decades and may remain clinically silent in the majority of people. In the coronary arteries, it remains asymptomatic until it progresses to cause a haemodynamically significant flow limiting lesion leading to symptoms of myocardial ischaemia. However, a substantial proportion of patients progress abruptly from inapparent disease to a myocardial infarction or possible death, due to thrombus formation following acute rupture or erosion of non-stenotic plaques.1,2 This highlights the importance of detecting atherosclerosis in the early phase of disease to facilitate effective disease modification strategies in predisposed individuals. In recent years, high-resolution ultrasound techniques have been developed extensively to detect early atherosclerotic changes. A generalised increase in common carotid artery (CCA) intima-media thickness (IMT) is an early manifestation of the atherosclerotic process and is readily measured by ultrasound imaging. This article discusses: (1) methodological aspects of ultrasound measurement of carotid IMT, (2) the determinants of carotid IMT, (3) the relationship of carotid IMT to coronary atherosclerosis, (4) the value of carotid IMT in predicting cardiovascular disease and (5) the potential role of carotid IMT in clinical practice as a means to stratify patients. In view of the scope of this article, the implications of detecting manifest carotid atherosclerotic plaque will not be specifically addressed.

CAROTID ULTRASOUND IMAGING

Using high resolution ultrasound imaging, carotid IMT assessment has emerged as a valuable tool for detecting and monitoring progression of atherosclerosis. The intima is the innermost layer of the arterial wall and consists of a single layer of endothelial cells in direct contact with blood, while the media consists mainly of smooth muscle cells constituting most of the thickness of the arterial wall with the adventitia making up the outermost layer which is mainly composed of collagen. Although ultrasound imaging cannot discriminate between the intimal and medial layers because of insufficient axial resolution, the intima—media complex can be identified as the double line density of the intimal—luminal and the medial—adventitial interfaces (figure 1). The validity of this ultrasound measurement of carotid IMT has been well established by comparison with histological specimens.3

METHODOLOGY OF CAROTID IMT MEASUREMENT

Although the general principles of carotid IMT measurement are common to all B-mode ultrasound studies, the methodologies used in these studies vary considerably with regard to image acquisition and analysis as shown in table 1. Measurements of IMT can be taken from the CCA, carotid bifurcation (bif) and internal carotid artery (ICA). Since the CCA is a tubular structure and is perpendicular to the ultrasound beam, measurement yield and reproducibility of IMT in this region are greater than for IMT measurements in the bif or ICA. Both near and far walls can be visualised on B-mode scans, but studies comparing ultrasound measurements with histology suggest that far wall carotid IMT measurements are more representative of the true thickness of the arterial wall.3 Near wall IMT measurements, in comparison, are limited by their dependence on the axial resolution and gain settings of the equipment used and hence are less accurate and reproducible. However, visualisation and reproducibility of near wall measurements, in particular, may be improved by the use of contrast ultrasound agents to enhance intimal border detection.4
Appendix VII

Abstract

Accepted for oral presentation in the AHA Congress, Dallas, November 2013.

Carotid Intima-Media Thickness is not related to Angiographic Coronary Artery Disease - Importance of Carotid Plaque Scores.

Author: Block, Safira; Babarishman Na’ir, Matthew Luckie, Univ. of Manchester, Manchester, United Kingdom; Kamal Khan, Univ Hosp of South Manchester NHS Fud Trust, Manchester, United Kingdom; Royal Malls, Univ of Manchester, Manchester, United Kingdom; Rajleeni K Khatkar, Royal Brompton and Harfield NHS Trust Hosp, London, United Kingdom.

Abstract: Introduction: Carotid intima-media thickness (CIMT) is considered to be a surrogate marker of atherosclerotic disease. Moreover, population studies have shown CIMT to be predictive of cardiovascular events. However, ultrasound methodology for measuring CIMT have varied and in some instances have incorporated measurement of manifest atherosclerotic plaque. As separate entities, the relationship of CIMT and carotid plaque with angiographic CAD and cardiovascular outcome is unclear.

Methods: Carotid ultrasound examination was performed in consecutive patients who underwent coronary angiography for evaluation of stable or acute chest pain. Mean, maximal CIMT and plaque measurements were made in accordance with the Maastricht consensus statement. CIMTmax represented the maximal plaque thickness. Carotid plaque was considered present if there was the absence of plaque was the maximal CIMT measurement.

Results: A total of 146 subjects were recruited into the study with a mean age of 59.9 ± 10.5 (range 29 to 85) years; 120 were men (83%) and 26 (18%) were women. Twenty-one percent of the study population had normal coronary angiography; 42% had stable CAD and 37% had presented with ACS. The table below shows a comparison of carotid parameters with extent of CAD.

Whereas CIMT measurements were similar in those with and without CAD, measurements incorporating carotid plaque (CIMTmax and plaque score) were significantly higher in the CAD groups. Spearman’s rank correlation analysis showed significant positive relationships of CIMTmax (r=0.32, p=0.001) and plaque score (r=0.42, p=0.001) with presence of CAD. Finally, combination of carotid parameters with CAD Syntax score showed a significant positive relationship with CIMTmax (r=0.22, p=0.008) only.

Conclusions: CIMT measurement is not associated with angiographic CAD, identifying carotid plaque and a composite score of CIMT with plaque (CIMTmax) may be more useful in risk stratification and prediction of coronary artery disease and cardiovascular events.

Table: Comparison of carotid measures with CAD

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<th>3+</th>
<th>4+</th>
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Category (Complete): 109. Non-Coronary Vascular Imaging (CT/MRI/Other)

Keyword (Complete): Carotid artery; Coronary artery disease; Plaque; Vascular disease
AHA congress Abstract

Introduction

Carotid intima-media thickness (CIMT) is considered to be a surrogate marker of atherosclerotic disease. Moreover, population studies have shown CIMT to be predictive of cardiovascular events. However, ultrasound methodologies for measuring CIMT have varied and in some instances have incorporated measurement of manifest atherosclerotic plaque. As separate entities, the relationship of CIMT and carotid plaque with angiographic CAD and cardiovascular outcome is unclear.

Methods

Carotid ultrasound examination was performed in consecutive patients who underwent coronary angiography for evaluation of stable or acute chest pain. Mean, maximal CIMT and plaque measurements were made in accordance with the Mannheim consensus statement. IMTmax represented the maximal plaque thickness or in the absence of plaque was the maximal CIMT measurement.

Results

A total of 146 subjects were recruited into the study with a mean age of 56.9 ± 10.6 (range 29 to 85) years; 120 were men (82%) and 26 (18%) women. Twenty-one percent of the study population had unobstruced coronaries, 42% had stable CAD and 37% had presented with ACS. The table below shows a comparison of carotid parameters with extent of CAD.
Whereas CIMT measurements were similar in those with and without CAD, measurements incorporating carotid plaque (IMTmax and plaque score) were significantly higher in the CAD groups. Spearman’s rank correlation analysis showed significant positive relationships of IMTmax ($r=0.32$, $p<0.001$) and plaque score ($r=0.42$, $p<0.001$) with presence of CAD. Finally, correlation of carotid parameters with CAD syntax score showed a significant positive relationship with IMTmax ($r=0.22$, $p=0.008$) only.

**Conclusions:** CIMT measurement is not associated with angiographic CAD. Identifying carotid plaque and a composite score of CIMT with plaque (IMTmax) may be more useful in risk stratification and prediction of coronary artery disease and cardiovascular events.
Appendix VIII

CIMT accreditation Certificate

Certificate of Accreditation

This is to certify that

Satheesh Nair

Has successfully completed training and accreditation in cIMT scanning for the Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AdDIT)

UCL, Institute of Child Health

Prof. John Deanfield

14/12/2008

Prof. John Deanfield