THE ROLE OF COMPUTED TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING IN PATIENTS WITH CARDIOVASCULAR DISEASE

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ABSTRACT

Background Although there have been recent advances, cardiovascular disease remains the commonest cause of premature death in the United Kingdom. There is a need to develop safe non-invasive techniques to aid the diagnosis and treatment of patients with cardiovascular disease.

Objectives The aims of this thesis are: (i) to establish whether coronary artery calcification can be measured reproducibly by helical computed tomography; (ii) to establish the effect of lipid lowering therapy on the progression of coronary calcification; (iii) to determine whether multidetector computed tomography can predict graft patency in patients who have undergone coronary artery bypass grafting; and (iv), to investigate the role of magnetic resonance imaging to assess plaque characteristics following acute carotid plaque rupture.

Methods In 16 patients, coronary artery calcification was assessed twice within 4 weeks by helical computed tomography. As part of a randomised controlled trial, patients received atorvastatin 80 mg daily or matching placebo, and had coronary calcification assessed annually. Fifty patients with previous coronary artery bypass surgery who were listed for diagnostic coronary angiography underwent contrast enhanced computed tomography angiography using a 16-slice multidetector computed tomography scanner. Finally, 15 patients with recent symptoms and signs of an acute transient ischaemic attack, amaurosis fugax or stroke underwent magnetic resonance angiography of the carotid arteries using dedicated surface coils. Plaque volume, regional plaque densities and neovascularisation were determined before and after gadolinium enhancement.

Results Quantification of coronary artery calcification demonstrated good reproducibility in patients with scores >100 AU. Despite reducing systemic inflammation and halving serum low-density lipoprotein cholesterol concentrations, atorvastatin therapy did not affect the rate of progression of coronary artery calcification. Computed tomography angiography was found to be highly specific for the detection of graft patency. Assessment of plaque characteristics by magnetic resonance scanning in patients with recent acute carotid plaque was feasible and reproducible.

Conclusions Coronary artery calcium scores can be determined in a reproducible manner. Although they correlate well with the presence of atherosclerosis and predict future coronary risk, there is little role for monitoring progression of coronary artery calcification in order to assess the response to lipid lowering therapy. Computed tomography can be used reliably to predict graft patency in patients who have undergone coronary artery bypass grafting, and is an acceptable non-invasive alternative to invasive coronary angiography. Magnetic resonance imaging techniques can be employed in a feasible, timely and reproducible manner to detect plaque characteristics associated with acute atherothrombotic disease.

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DECLARATION

This thesis represents research undertaken primarily in the Department of Cardiology at the Royal Infirmary of Edinburgh, and also at the Department of Clinical Neurosciences at the Western General Hospital, Edinburgh and the Radiology Department at the Borders General Hospital, Melrose. The substantial part of the work described has been my own and carried out during the period between 2003 and 2005 whilst I was a research fellow in Cardiology. The work has been published in peer reviewed journals; see bibliography. The thesis has not been accepted in any previous applications for a degree and all sources of information have been acknowledged.

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ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
AVERT	Atorvastatin Versus Revascularisation Treatment
AU	Arbitrary units
BELLES	Beyond Endorsed Lipid-Lowering with EBCT Scanning
CAC	Coronary artery calcification
CARE	Cholesterol And Recurrent Events
CE-MRA	Contrast-Enhanced Magnetic Resonance Angiography
CHD	Coronary heart disease
CRP	C-reactive protein
CT	Computed tomography
CTA	Computed tomography angiography
EBCT	Electron beam computed tomography
ECG	Electrocardiogram
fMRI	Functional magnetic resonance imaging
FOV	Field of view
FSPGR	Fast spoiled gradient recalled echo
HDL	High density lipoprotein
HMGCoA	3-hydroxy-3-methylglutaryl coenzyme A
HPS	Heart Protection Study
hsCRP	High sensitivity C-reactive protein
HU	Hounsfield units
IMA	Internal mammary artery
IVUS	Intravascular ultrasound
LDL	Low density lipoprotein
LIPID	Long-term Intervention with Pravastatin in Ischaemic Disease
MDCT	Multidetector computed tomography
MMPs	Matrix metalloproteinases
MRI	Magnetic resonance imaging
MRS	MR spectroscopy
NPV	Negative predictive value
ORION	Outcome of Rosuvastatin treatment on carotid artery atheroma: a magnetic resonance Imagin ObservatioN
PACC	Prospective Army Coronary Calcium
PPV	Positive predictive value
PROVE-IT	PRavastatin Or atorVastatin Evaluation and Infection Trial
RECALL	Risk Factors, Evaluation of Coronary Calcium and Lifestyle
REVERSAL	Reversal of Atherosclerosis with Aggressive Lipid Lowering
SALTIRE	Scottish Aortic stenosis Lipid-lowering Therapy, Impact on Regression
SD	Standard deviation
SE	Standard error
SPECT	Single Photon Emission Computed Tomography
TNT	Treating New Targets
TOF	Time of flight
U	Units
USPIO	Ultrasmall superaramagnetic iron oxide
WOSCOPS	West of Scotland Coronary Prevention Study Group

CHAPTER 1

INTRODUCTION:

THE ROLE OF COMPUTED TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING IN PATIENTS WITH CARDIOVASCULAR DISEASE

1.1 BACKGROUND

Although there have been recent advances in diagnosis and treatment, cardiovascular disease is the commonest cause of premature death in the United. Coronary heart disease (CHD) accounts for over 64, 000 deaths in men and 33,000 deaths in women in the UK annually (WHO mortality statistics 2002) which equates to over 22% of all deaths in men and 17% in women. Cerebrovascular disease accounts for over 25,500 deaths in men and nearly 42,000 deaths in women each year (WHO mortality statistics). Overall, diseases directly attributable to arterial disease accounted for 31% of all deaths in the UK in 2002.

The principal initiating event in the pathogenesis of acute cardiovascular events is rupture or erosion of the atherosclerotic plaque (Davies 2000). This leads to intravascular thrombus formation and the potential for acute vessel occlusion or thromboembolism. Clinically, this is most dramatically manifest by the onset of acute myocardial infarction or stroke.

1.2 ATHEROSCLEROSIS

1.2.1 Natural History

Atherosclerotic lesions occur predominantly in medium-sized muscular arteries, including coronary, renal, carotid, basilar, and vertebral arteries. Such lesions are categorized according to histological characteristics (Table 1.1; AHA recommended classification of atherosclerotic plaque, Fig. 1.1 (Stary, Chandler et al. 1995)).

Table 1.1 AHA recommended classification of atherosclerotic plaque (Stary, Chandler et al. 1995).

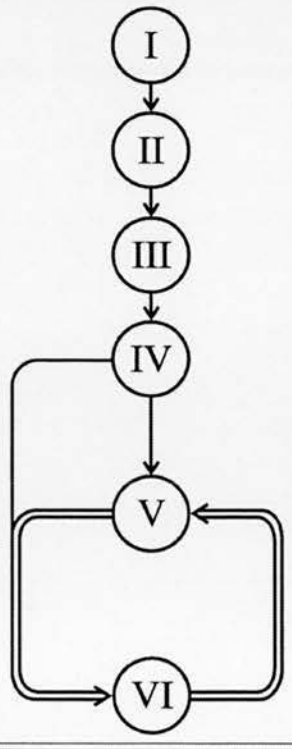
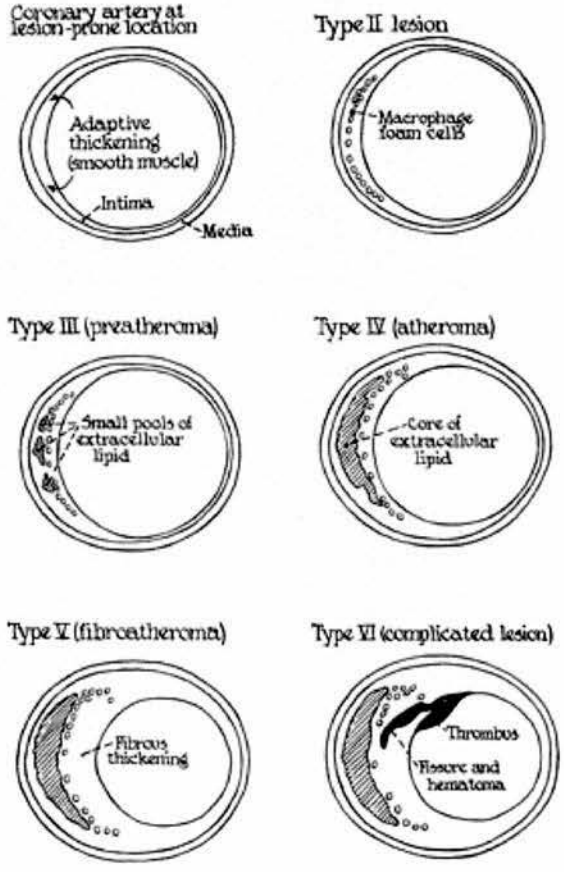
Nomenclature and main histology	Sequences in progression	Main growth mechanism	Earliest onset	Clinical correlation
Type I (initial) lesion isolated macrophage foam cells		growth mainly by lipid accumulation	from first decade	clinically silent
Type II (fatty streak) lesion mainly intracellular lipid accumulation				
Type III (intermediate) lesion Type II changes & small extracellular lipid pools			from third decade	
Type IV (atheroma) lesion Type II changes & core of extracellular lipid				clinically silent or overt
Type V (fibroatheroma) lesion lipid core & fibrotic layer, or multiple lipid cores & fibrotic layers, or mainly calcific, or mainly fibrotic		accelerated smooth muscle and collagen increase	from fourth decade	
Type VI (complicated) lesion surface defect, hematoma-hemorrhage, thrombus		thrombosis, hematoma		

Fig. 1.1 Plaque types (Stary, Chandler et al. 1995).



The disease process is one which begins in childhood, with an increase in the numbers of intimal macrophages and macrophage foam cells (Type I lesion). This progresses to the development of fatty streaks (Type II lesions), which have predominantly intracellular lipid collections. From the third decade, isolated extracellular lipid pools begin to appear (Type III lesions). Type I-III lesions are small and do not result in any clinical manifestations, nor do they cause any luminal narrowing.

Lesions in highly susceptible sites of the vascular tree exhibit higher macrophage numbers, a greater lipid content, and have an increased propensity towards progression.

These susceptible sites occur throughout the coronary circulation, renal arteries and internal carotid artery at the level of the carotid sinus, and are the result of altered mechanical stress, primarily at branching points. These altered mechanics lead to a process known as adaptive intimal thickening, whereby the artery adjusts its inner contour through localized changes in the thickness of the intima in an attempt to maintain normal conditions of flow. As early changes evolve, the lipid pools increase in size to form a confluent lipid core (Type IV lesion). Type IV lesions rarely cause any significant luminal obstruction unless blood lipid levels are very high, leading to a rapid accumulation of lipid in the core. At this stage, the tissue layer between the lipid core and the endothelium is recognisable histologically as normal intima. As tissue is disrupted by lipid accumulation, smooth muscle cells hypertrophy and collagen is laid down to form the fibrous cap, which is characteristic of type V lesions. This fibrous connective tissue formation is a reparative response to the injury caused by the disruption of normal intercellular matrix by extracellular lipid deposition. The formation of new layers initially results in expansion of the vessel wall in order to maintain adequate lumen area (Glagov, Weisenberg et al. 1987). Capillaries at the periphery of the lipid core may be large and numerous, and this capillary network may extend into the newly formed fibromuscular cap. The extent of such neovasculature is thought to correlate with plaque vulnerability (Kerwin, Hooker et al. 2003). Type V lesions may have differing compositions, and a subclassification represents these variations. Type Va lesions are known as multilayered atheroma, with several lipid cores interspersed with layers of fibrous connective tissue. These may result from alterations in vascular haemodynamics caused by luminal narrowing and also by disruption of lesion surface with fibrosis of

resultant haematoma and thrombi deposits. Type Vb lesions contain calcium deposits, and in type Vc lesions, fibrosis predominates with a relative paucity of lipid. Type VI lesions are a result of surface disruptions, haematoma or thrombosis superimposed upon a type IV or V lesion. These lesions are termed *complicated lesions* and are subclassified into Type VIa, surface disruption, Type VIb, haematoma or haemorrhage, and Type VIc, thrombosis.

1.2.2 Calcium Deposits in Atherosclerotic Lesions

Type I-II lesions develop during childhood and puberty, and are composed of lipid-laden macrophages and, later, lipid pools. As the lipid core expands, replacing the intima, calcium granules appear in the cytoplasm of smooth muscle cells. As these cells apoptose and break up, the calcium becomes extracellular and forms aggregates, which are distributed throughout the lipid core. Calcification of cell components also occurs following apoptosis, further contributing to calcium load. As plaques progress, typically around the fifth decade of life, calcific deposits can become confluent, with fusion of adjacent aggregates to form lumps or plates of calcium. It has been suggested that arterial calcification may represent an attempt to stabilize the atherosclerotic endothelium, by forming a protective layer. Intravscular ultrasound (IVUS) studies have shown that calcified lesions are relatively more stable than their non-calcified counterparts (Mintz, Popma et al. 1995). Indeed, a plaque with a heavily calcified cap is around five times stiffer than a non-calcified lesion, and resistant to rupture (Lee, Grodzinsky et al. 1991). However, the interface between a calcified section and a non-calcified section can be a site prone to rupture, particularly in the context of post-

angioplasty dissection, due to increased stress near the junction of the calcific plaque and the adjacent intima. It is thought, however, that with time and more extensive calcification, these weak points become less vulnerable, which would explain why, in general, calcification is not thought to be an indicator of a plaque which is at high risk of rupture.

1.2.3 Vulnerable Plaque

Although luminal narrowing by atherosclerotic plaques is responsible for some of the clinical manifestations of the disease, it is plaque disruption and subsequent occlusive thrombus formation that causes the most serious acute clinical manifestations, namely acute ischaemic symptoms of stroke, myocardial infarction and sudden death. Observational studies have shown that the majority of culprit lesions have <70%, and often <50%, luminal stenosis (Giroud, Li et al. 1992). The reason being that, in addition to plaque morphology, positive versus negative remodeling has an important role to play in determining the overall effect on lumen size. The greater outward remodeling, first described by Glagov et al (Glagov, Weisenberg et al. 1987), of unstable or vulnerable plaques may minimize luminal compromise despite large plaque volume. IVUS studies support this and have shown a correlation between outward, or positive remodeling and unstable clinical presentation, whereas inward, or negative remodeling is more common in stable angina (Schoenhagen, Ziada et al. 2000).

Rupture-prone plaques have been shown to have different histomorphological features to stable intact plaques. So called vulnerable plaques tend to be large, exhibiting positive

remodeling, with a large lipid core (occupying more than 40% of the plaque volume), a thin fibrous cap which is depleted of smooth muscle cells and collagen and therefore easily disrupted or fissured. In addition, they are characteristically accompanied by numerous macrophages and inflammatory cells at the cap surface (Libby 2001). The smooth muscle cell population also has a bearing on plaque stability, and sites of plaque rupture leading to fatal occlusive thromboses typically have few smooth muscle cells (Davies, Richardson et al. 1993). The structural component of the fibrous cap includes collagen, elastin and proteoglycans which are derived from smooth muscle cells. The cap forms a protective barrier between the circulating blood and the highly thrombogenic lipid core, but this thins out in the vicinity of the rupture. Vulnerable plaques frequently show increased neovascularity (Tenaglia, Peters et al. 1998) which may provide a source for recruitment of inflammatory cells into the plaque.

Inflammatory cytokine dynamics also have a major bearing on plaque stability. Depletion of matrix components from the fibrous plaque leads to cap thinning and predisposes the cap to rupture, either spontaneously or in response to haemodynamic or other triggers. Enhanced matrix breakdown has been attributed to a family of matrix-degrading metalloproteinases (MMPs) that are expressed by inflammatory cells (Brown, Hibbs et al. 1995; Rajavashisth, Xu et al. 1999) and activated by inflammatory cytokines, reactive oxygen species and haemodynamic stress, all of which are components existing in the atherosclerotic plaque and its surrounding environment (Rajavashisth, Xu et al. 1999).

1.3 DIAGNOSTIC CHALLENGES

With advances in technology, techniques for identification and quantification of atherosclerotic plaque disease have improved immeasurably over the past decade. In addition, improved pharmaceutical interventions with which to aid the prevention, and reduce progression, of atherosclerotic disease has placed even more emphasis on identifying 'at-risk' patients. Catheter-based procedures give some information as to the morphological and haemodynamic characteristics of atherosclerotic plaque, but image only the lumen and carry the risk of an invasive procedure. There is a need to develop techniques with which to image vessels non-invasively both to aid diagnosis and treatment, and to minimise risks associated with invasive imaging such as coronary angiography. Non invasive imaging techniques provide the opportunity to detect lesions before symptoms occur, to detect so called vulnerable plaques and, potentially, to monitor disease progression.

1.3.1 Computed Tomography

Computed tomography (CT) was invented in 1972 by the British engineer Godfrey Newbold Hounsfield at THORN EMI Central Research Laboratories, and independently by Allan McLeod Cormack of Tufts University, Massachusetts. The idea was conceived in 1967, and publicly announced in 1972. In 1979, they were jointly awarded the Nobel Prize for Medicine, and Hounsfield later honoured with Knighthood in England for his contributions to medicine and science.

The original prototype took 160 parallel datasets through 180 angles, each 1° apart. The scan duration was over five minutes, and the processing time a further two and a half hours by algebraic reconstruction techniques. The first clinical scanners became available between 1974 and 1976 and were limited to imaging of the brain only. The scanner involved the use of a water filled Perspex tank with a pre-shaped rubber head piece which enclosed the patient's head, in order to reduce the dynamic range of the radiation reaching the detectors. The resolution of early scanners was relatively low, having a matrix of only 80 x 80 pixels. Whole body systems, however, soon became available and were widely used in clinical practice by 1980.

First generation scanners used a single pencil beam of radiation and a single detector. The fixed source/detector gantry worked on a translate-rotate basis, rotating in 1° increments and taking a total of 3-5 minutes for a single slice. Second generation scanners used a narrow fan shaped x-ray beam falling on a small curved array of detectors, allowing the translation and rotation to be increased to 30°, thereby requiring fewer angular steps and reducing the scan time to around 20 seconds per slice. Third generation scanners were faster again, with a wider fan shaped beam of x-rays being directed towards an array of detectors that are in a fixed position relative to the x-ray source. This eliminated the need for the time consuming translation phase by simply rotating continuously through 360° round the patient. This reduced the scan time to 10 seconds per slice, thereby making imaging of, for example, chest or abdomen practicable, where it had previously been limited to the head. In fourth generation scanners, the x-ray tube alone rotates round the patients who is positioned in a stationary 360° ring of

detectors and is equivalent to third generation scanners in terms of speed of scanning. The use of slip-ring technology has replaced the spooled cable technology of older CT scanners, allowing indefinite rotation of the tube, making possible helical scanning. Further increase in scan speed is possible by use of multidetector row CT systems (MDCT), whereby multiple stationary detector rings allow multiple slices to be scanned simultaneously. MDCT permits reconstruction of images in planes other than axial, and has made possible the recent advances in cardiac imaging and CT angiography.

CT offers the potential to provide high definition images of the heart and has the benefit of being non-invasive. Electron-beam CT (EBCT) has been developed and used almost exclusively for imaging the heart, due to its high temporal resolution which is required to minimise movement artefact associated with the beating heart. EBCT employs a rapidly rotating electron beam which is focussed on a stationary tungsten target, producing temporal resolution of 50-100 ms. The majority of published cardiac imaging data has been performed using EBCT, but recent technological advances in MDCT have enabled the simultaneous acquisition of multiple overlapping sections per gantry rotation. This, in addition to increased gantry rotation speed, has resulted in high temporal and spatial resolution cardiac imaging in a single breath hold (Fuchs, Kachelriess et al. 2000). While EBCT has, until recently, had the advantage of better temporal resolution, MDCT offers a higher signal-to-noise ratio, shorter scan time and higher spatial resolution.

1.3.2 Magnetic Resonance Imaging

Felix Bloch, from Stanford University, and Edward Purcell, from Harvard University discovered the magnetic resonance phenomenon independently in 1946. They were jointly awarded the Nobel prize for physics for their discovery in 1952. Between 1950 and 1970, it was developed and used for chemical and physical molecular analysis. In 1971, Raymond Damadian, a medical doctor at the State University of New York in Brooklyn, showed that the nuclear magnetic relaxation times of tissues and tumours differed, leading to the idea that the technique may be used as a means of detecting cancer. Magnetic resonance imaging (MRI) was first demonstrated on small test tube samples in 1973 by Paul Lauterbur, a Professor of Chemistry at the State University of New York at Stony Brook, and described in the seminal paper entitled 'Image formation by induced local interaction; examples employing magnetic resonance', published in Nature (LAUTERBUR 1973). Lauterbur described a new imaging technique which he termed zeugmatography, referring to the joining together of a weak gradient magnetic field with the stronger main magnetic field allowing the spatial localisation of two test tubes of water. He used a back projection method to produce an image of the two test tubes. In 1975, Richard Ernst, from Zurich, proposed magnetic resonance imaging using phase and frequency encoding and the Fourier Transform which provides the basis of current MRI techniques. Peter Mansfield, who led a nuclear magnetic resonance research group in Nottingham, further developed the utilisation of gradients in the magnetic field, and demonstrated mathematical analysis of the signals in order to develop a useful imaging technique. In 2003, the Nobel Prize for Physiology and Medicine was awarded

to Paul Lauterbur and Peter Mansfield for their discoveries concerning magnetic resonance imaging.

The first commercial scanner in Europe was installed in 1983 in the Department of Diagnostic Radiology at the University of Manchester Medical School. Since then, further applications have been developed, including echo-planar imaging, used to perform real-time movie imaging of a single cardiac cycle, magnetic resonance angiography, allowing imaging of flowing blood without the use of contrast agents, and functional MRI (fMRI) which allows mapping of the function of the various regions of the human brain. The past twenty years have seen MRI become a widely used diagnostic imaging technology and, with new techniques and ever more powerful machines emerging, the speed and precision of MRI has increased dramatically. It is likely to supersede conventional catheter angiography as the imaging modality of choice in vascular disease, and provides a highly flexible non-invasive alternative in vascular and cardiac imaging as well as offering information on function.

Magnetic resonance imaging is based upon the radiofrequency signal from protons following the administration of a radiofrequency pulse. The emitted signal varies as to the water content and relaxation times (T1 and T2), with signal intensities on an MR image reflecting the biochemical environment of protons in the tissue of interest. Experimental evidence has shown that MRI may be a useful tool for imaging both native coronary arteries and bypass graft vessels. However, this is technically demanding due to the small size of the vessels, their complex three dimensional anatomy and their constant

motion due to cardiac and respiratory variation. MR is used with greater success for evaluation of the peripheral vasculature. In addition to detecting stenosis or occlusion of an artery, MRI can be used to assess the vessel wall directly for atherosclerotic changes and, by way of plaque characterisation, may help identify patients at high risk of cardiac events.

1.4 THERAPEUTIC STRATEGIES

The association between raised plasma cholesterol and cardiovascular risk is well-established. Statin therapy has a proven role in the primary (Shepherd, Cobbe et al. 1995; Downs, Clearfield et al. 1998) and secondary (4S trial investigators, 1994; LIPID trial investigators, 1998; Lewis, Moye et al. 1998; Heart Protection Study trial investigators, 2002) prevention of cardiovascular disease. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are the most potent class of drugs available for the treatment of hypercholesterolaemia, accounting for 96% of all drugs used in the treatment of this condition. The beneficial effect of lipid-lowering upon morbidity and mortality in high risk subjects in WOSCOPS (40 mg pravastatin) (Shepherd, Cobbe et al. 1995) as well as those with established coronary artery disease is validated in large population-based studies including 4S (using 20-40 mg simvastatin), CARE and LIPID (both using 40 mg pravastatin) (4S trial investigators, 1994; LIPID trial investigators, 1998; Lewis, Moye et al. 1998) where 25-35% reductions in LDL-cholesterol led to 24-37% fewer fatal and non-fatal myocardial infarctions. The Heart Protection Study (HPS) showed that even patient populations with low serum cholesterol concentrations (35% with no previous coronary disease) benefited from statin therapy (simvastatin 40 mg)

with a reduction in cardiovascular mortality of 17% and in all vascular events of 24% (Heart Protection Study Investigators, 2002). A recent meta-analysis of 14 randomised trials of statins showed that an overall reduction of around one fifth per mmol/L LDL cholesterol reduction equated to a reduction by 48 per 1000 major cardiovascular events in those with pre-existing CHD, and 25 per 1000 amongst those with no disease at baseline (Baigent, Keech et al. 2005). The mechanisms whereby lipid-lowering might act beneficially include atheromatous plaque regression and stabilisation (Jukema, Bruschke et al. 1995; Rosenson and Tangney 1998) and attenuation of coronary endothelial dysfunction (Treasure, Klein et al. 1995). Statins may also directly modify atherosclerosis independent of cholesterol lowering effects through additional pleiotropic effects.

Despite effective lipid-lowering therapy in several primary and secondary prevention trials with statins, most patients continue to experience coronary and cerebrovascular events. The PROVE-IT trial demonstrated that patients who achieve a reduction in LDL-cholesterol to <70 mg/dl and hsCRP <2mg/l have the lowest rates of recurrent myocardial infarction and cardiovascular death amongst statin-treated patients. However, the study also demonstrated that current aggressive lipid-lowering regimens (atorvastatin 80 mg), whilst achieving superior reduction in LDL-cholesterol, were not sufficient to bring the majority of patients to below the threshold required to maximise benefit (Ridker, Morrow et al. 2005). The Treating New Targets study (TNT) demonstrated that aggressive lipid-lowering with atorvastatin 80 mg was associated with a reduction in cardiovascular events compared with standard LDL-cholesterol lowering in patients with acute coronary

syndrome, but at the expense of an increased incidence of adverse drug reactions (LaRosa, Grundy et al. 2005).

Clinical and basic experimental studies suggest that aggressive lipid-lowering may also improve acute and long-term clinical outcomes by acting to stabilise plaque and inducing plaque regression, ultimately decreasing total plaque burden. In the Atorvastatin Versus Revascularisation Treatment (AVERT) study, a randomised study investigating aggressive lipid lowering versus intervention, 341 patients with stable single vessel disease who received 80 mg of atorvastatin to achieve a mean serum LDL-cholesterol of 2.0 mmol/l had a lower incidence of combined ischaemic events compared to treatment with coronary angioplasty.(Pitt, Waters et al. 1999) This study suggested that aggressive reduction in lipid levels is more likely to prevent further progression of minimal coronary artery lesions than angioplasty by helping to stabilise atherosclerotic plaques, thereby preventing plaque rupture and reducing the incidence of ischaemic events.

Experimental models suggest that statins foster plaque stability through a reduction in macrophage cholesterol ester content and increased smooth muscle cell proliferation and collagen production. The thrombotic sequelae of plaque disruption are mitigated by statins through inhibition of platelet aggregation and maintenance of a favourable balance between pro-thrombotic and fibrinolytic mechanisms (Takemoto and Liao 2001). Statins reduce progression of atheromatous plaque and new lesion formation in subjects with elevated LDL-cholesterol (Multicentre Atheroma Study (MAAS) 1994; Jukema, Bruschke et al. 1995; Pitt, Mancini et al. 1995). In the coronary circulation, the Reversal

of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial compared the effect of moderate lipid lowering using 40 mg pravastatin with aggressive lipid lowering using 80 mg atorvastatin upon the progression of atheroma. There was a significant difference in the primary endpoint of the percentage change in atheroma volume, with an increase of 2.7% with pravastatin compared to -0.4% with atorvastatin ($p<0.05$). An additional anti-inflammatory effect has been postulated, and is supported by a greater reduction in CRP (36% with atorvastatin compared with 5% with pravastatin) and atherogenic lipoproteins in the atorvastatin group (Nissen, Tuzcu et al. 2004). It was not, however, powered to examine clinical events. The PROVE-IT (Pravastatin or Atorvastatin Evaluation and Infection Therapy) Trial showed that intensive lipid lowering with atorvastatin 80 mg reduced the cardiovascular clinical event rate in patients recently hospitalised with acute coronary syndrome when compared with standard lipid lowering with pravastatin 40 mg (Cannon, Braunwald et al. 2004). The benefit emerged as early as 30 days, and was consistent over time, suggesting that the population of patients with acute coronary syndromes, who have a culprit lesion and frequently multiple additional vulnerable plaques, can derive particular benefit from early and intensive lipid lowering with statins. The current evidence base confirms the benefit of statin therapy irrespective of baseline LDL-cholesterol levels and there is a compelling case for aggressive lipid lowering in all patients with known vascular disease.

1.5 SCREENING AND DIAGNOSTIC TOOLS

1.5.1 Coronary Artery Calcium Scoring

The presence of coronary artery calcification is synonymous with atheromatous plaque (Frink, Achor et al. 1970; Rifkin, Parisi et al. 1979). Frink et al originally compared the association between coronary artery calcification (CAC) and occlusive coronary artery disease on radiographic and pathologic studies, and showed a correlation between the degree of calcification and the presence of significant narrowing (Frink, Achor et al. 1970). Since then, there have been numerous studies confirming this association.

CAC is an independent risk factor for coronary heart disease, with even low coronary calcium scores at computed tomography doubling the risk of coronary events.(Pletcher, Tice et al. 2004) The extent of arterial calcification correlates closely with atherosclerotic burden, (Sangiorgi, Rumberger et al. 1998) and a high coronary calcium score at computed tomography denotes a very high risk of future coronary events (Wayhs, Zelinger et al. 2002). Recently published research has shown that coronary calcium score is an accurate predictor of coronary artery events independent of standard risk factors (Arad, Goodman et al. 2005). Because CAC is an easily detected surrogate for atherosclerotic plaque disease, it is a potential tool for predicting cardiac risk. Indeed, CAC detection as a way of screening for the presence of asymptomatic coronary artery disease and is being used increasingly in centres around the world.

One method of detecting of coronary calcification is by CT imaging. Previously, conventional and spiral CT exhibited poor spatial and temporal resolution, and much of

the published research into this modality has used EBCT, a modality which is not readily available in routine clinical practice in the UK. EBCT imaging involves acquisition of 30-40 images over the length of the myocardium, each slice having a fixed thickness of 3 mm with the entire coronary tree being imaged in a single breath hold (20-30 seconds). EBCT has been shown to allow easy quantification of CAC load, but at the expense of poor reproducibility (interscan variability between studies varying from 13-38% (Shields, Mielke et al. 1995; Callister, Cooil et al. 1998; Achenbach, Ropers et al. 2001)). Not only does this degree of variability devalue the absolute score, it also jeopardises the ability to detect any changes in the score over time. However, the latest generation of MDCT scanners (16-slice and 64 slice), have improved temporal and spatial resolution. This has led to improved accuracy and reproducibility, and favourable comparison between spiral CT and EBCT (Carr, Crouse et al. 2000; Kopp, Ohnesorge et al. 2002). Kopp et al (Kopp, Ohnesorge et al. 2002) demonstrated that, when compared with EBCT, MDCT allows accurate and repeatable measurements of CAC to be obtained, with low interscan variability.

Quantification of coronary artery calcium using CT was first described by Agatston et al (Agatston, Janowitz et al. 1990) in 1990. A threshold of 130 Hounsfield units (HU) was used to denote the presence of calcium, and a coronary calcium score was determined by placing a region of interest around all calcified lesions within a coronary artery (Fig 1.2). A total score was determined by adding up the scores for each slice. This method has been reproduced throughout the literature and the scores have been used as a predictor of risk. A metanalysis of relevant articles calculated that a coronary artery calcium score of

between 1 and 100 equates to an adjusted relative risk of 2.1 (Pletcher, Tice et al. 2004). There is evidence to suggest that the coronary calcium score may be used as a predictor of risk in asymptomatic patients(Pletcher, Tice et al. 2004) but, until recently, little conclusive evidence to support its use in monitoring disease progression or response to therapy (Arad, Spadaro et al. 2005; Raggi, Davidson et al. 2005).



Fig. 1.2 Smartscore™, a program used to calculate CAC score by drawing a region of interest around all calcified lesions in each coronary artery (GE Healthcare, www.gehealthcare.com)

1.5.2 Computed Tomography Angiography

Whilst CAC is indicative of atheroma and may be a useful screening tool, it provides no information on stenosis severity hence the need for invasive imaging modalities. The gold standard method of assessing coronary arterial patency is coronary angiography. Life-threatening complications are rare (approx. 1 in 1000), but include MI, stroke, aortic or coronary dissection, cardiac rupture, air embolism, arrhythmia and peripheral vascular complications such as haemorrhage, infection or limb ischaemia. Other complications are more common and include haematoma at puncture site, angina, vasovagal reactions and allergies to contrast agents. The assessment of coronary arterial patency in a non-invasive and readily available manner would have major benefits for the management and treatment of patients with coronary artery disease.

CT angiography (CTA) is a technique which can be performed rapidly and safely for the assessment of vascular disease. With the advent of MDCT, excellent image quality is now possible with higher resolution than was previously achieved with single detector row technology. Current MDCT scanners obtain up to 16 simultaneous helices, and 64-slice scanners are now becoming available. CTA has several advantages over conventional angiography, the foremost being that it is less invasive. In addition, it permits three-dimensional volumetric reconstructions, allowing visualisation of the anatomy from multiple angles and in multiple planes after a single acquisition. It also allows visualisation of surrounding soft tissues and adjacent anatomic structures, and is more cost effective than conventional angiography and traditional methods of

investigation such as exercise ECG and stress echo (Bluemke and Chambers 1995; Dewey and Hamm 2007). Coronary CTA is typically performed in a spiral scan mode, permitting three-dimensional scanning of the heart during a single breath hold. Data are retrospectively gated to the electrocardiogram to reconstruct the images during the diastolic phase of the cardiac cycle. Reconstruction is possible with slice thickness as little as 0.75 mm and a spatial resolution of approximately 0.4 x 0.4 mm. Patient radiation dose at MDCT is typically in the region of 11.0±1.9 for 16-slice CTA and 11±4.1 mSv for 64-slice CTA (Hausleiter, Meyer et al. 2006).

Intravascular contrast enhancement is employed in order to differentiate vessel lumen from vessel wall at CT. This allows both angiographic evaluation of the coronary artery lumen and characterisation of the coronary artery wall. Reconstruction algorithms allow the vessels to be displayed at various axes in order to follow the course of individual coronary arteries (Fig. 1.3). Technology also permits display of three dimensional volume rendered images which, whilst allowing assessment of spatial orientation, provide limited information about the arterial lumen and vessel wall (Fig. 1.4). The sensitivity and specificity for detection of significant luminal stenoses (>50%) with the current 16 slice MDCT, when measures are taken to ensure adequate heart rate control of around 60 beats per minute, is around 90% (Nieman, Cademartiri et al. 2002; Ropers, Baum et al. 2003; Mollet, Cademartiri et al. 2004).

Fig. 1.3 CT coronary angiogram; multiplanar reconstruction algorithm

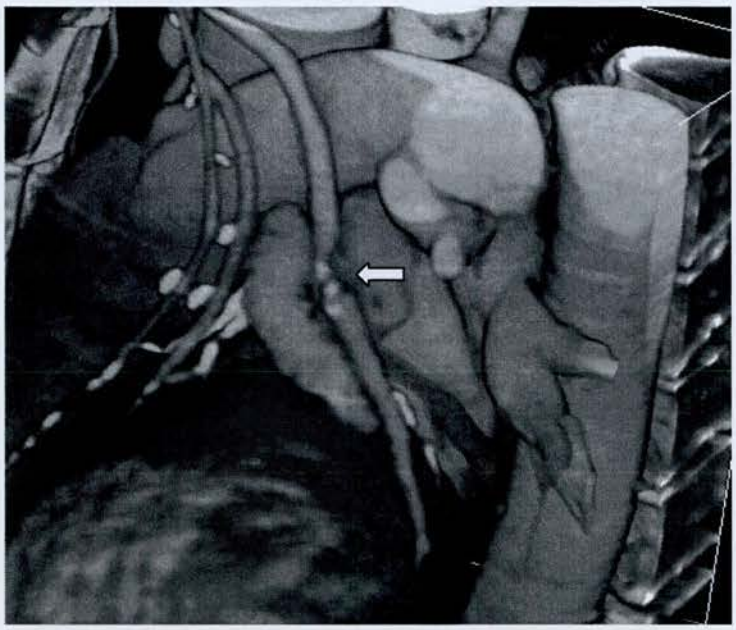


Fig. 1.4 CT coronary angiogram; Volume rendered image



MDCT is a feasible alternative to invasive coronary angiography in patients with a stable heart rhythm and who are able to co-operate with the required 20 second breath hold. However, it is of limited value in those with irregular heart rhythms, and atrial fibrillation is a contraindication. Frequent extrasystoles may also degrade image quality due to interference with ECG gated reconstruction. Rapid heart rates and inadequate breath holding during the scan may also contribute to motion artefact. Large depositions of calcium in the vessel wall interfere with image quality, causing partial voluming and beam hardening which can completely obscure the cross section of the vessel and prevent assessment of the patency of the coronary artery lumen. Similarly effects, metal objects such as stents, surgical clips, and sternal wires can also interfere with assessment of the vessel. Finally, even in co-operative patients with stable heart rates, coronary artery motion artefacts may still interfere with image quality, in particular in the assessment of the right coronary artery, the most rapidly moving segment and the site of most motion artefacts.

The large calibre and more static location of bypass grafts make them particularly suitable for investigation by potential non-invasive imaging modalities such as MDCT angiography. Published data have reported sensitivity and specificity of up to 100% and 99.4% respectively for the detection of graft patency in the immediate post-operative period (Song, Ito et al. 2005). This study, however, looked at patients immediately post-coronary artery bypass operation, who may be expected to have a high patency rate and no graft vasculopathy, thereby minimising any ambiguity caused by poor flow in chronically diseased grafts. Recent studies looking specifically at graft patency outwith

the immediate postoperative period have reported sensitivities of 90-100% and specificities of 88-100% for detection of graft patency (Achenbach, Moshage et al. 1997; Engelmann, von Smekal et al. 1997; Hoshi, Yamauchi et al. 2001; Marano, Storto et al. 2004) and as good as 100% and 97% respectively for the detection of significant stenoses (Achenbach, Moshage et al. 1997).

Whilst there have been many modest studies assessing the ability of CTA to detect vessel patency and stenosis, the clinical value of MDCT is yet to be established in large multicentre studies, evaluating populations with differing prevalence, presentation and extent of coronary arterial disease. Until this is achieved, conventional invasive angiography remains the gold standard method of assessing coronary artery disease.

1.5.3 Magnetic Resonance Angiography

Acute cardiovascular events, particularly acute coronary syndromes and stroke, are a consequence of atherosclerotic plaque rupture. Coronary angiography provides information regarding stenosis severity, but not plaque volume or morphology. Luminal narrowing is an indirect measure of plaque size and may underestimate the true plaque burden. Glagov et al described the phenomenon of compensatory enlargement, whereby the coronary arteries enlarge in relation to plaque area such that functionally significant luminal stenoses may not be evident until the plaque occupies up to 40% of the internal elastic lamina area (Glagov, Weisenberg et al. 1987). In addition, as discussed earlier, IVUS studies have shown a correlation between outward, or positive remodeling and unstable clinical presentation (Schoenhagen, Ziada et al. 2000). This emphasises the

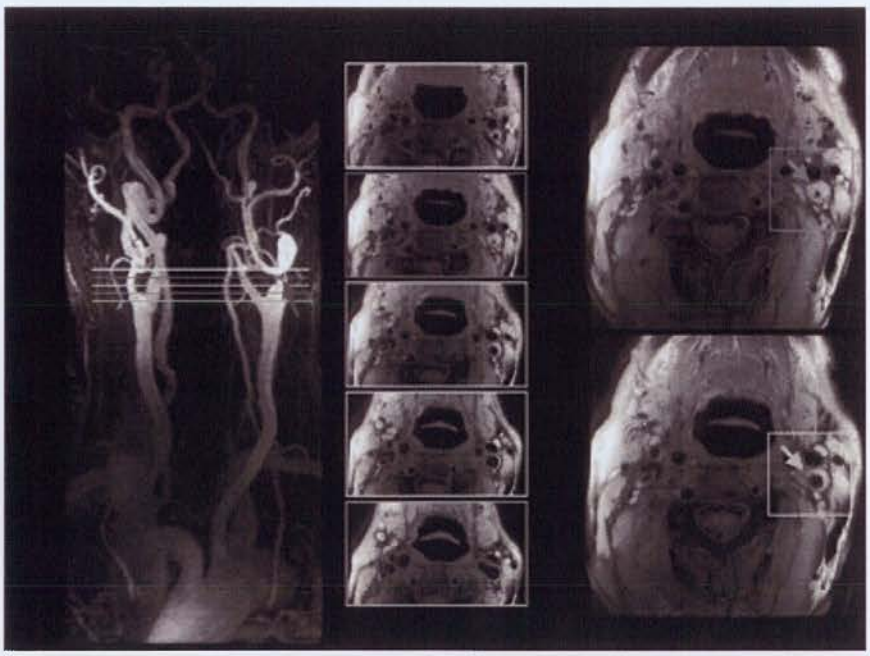
importance of imaging modalities which can identify the inner and outer adventitial margins.

High resolution imaging of coronary arteries is currently only possible with invasive techniques, such as IVUS (Newby, McLeod et al. 2001; McLeod, Newby et al. 2003). IVUS imaging of complex atherosclerotic plaques is limited by the poor resolution of *in situ* thrombus, potential distortion of geometry and the inability to image though calcified plaques. Non-invasive techniques, such as MRI, hold promise, but applicability to the coronary circulation is limited by the relatively small calibre of the coronary vasculature and the movement artefact induced by continuous myocardial contraction. Carotid arteries are more suitable for non-invasive imaging because of their greater calibre, superficial location and relatively static position. This vessel, therefore, lends itself more readily to the assessment of acute plaque events and subsequent remodelling. MRI provides a modality of non-invasive imaging without the need for ionising radiation and, with the use of dedicated surface coils, can produce high-resolution images of carotid atherosclerotic plaques.

MRI differentiates plaque components based upon differences in their chemical composition, water content, physical state, molecular motion of diffusion. It involves subjecting the patient to a high local magnetic field which aligns the protons in the body; these protons are excited by a radiofrequency pulse and subsequently detected by receiver coils. The ability to obtain images of the atherosclerotic vessels is dependent upon the amount of available signal, contrast and the lack of noise. Using dedicated surface coils,

images of the carotid artery can be obtained with resolution of 0.4 x 0.4 x 3 mm. The images can be 'weighted' to the T1, T2 or proton density values which emphasises different properties and is useful to differentiate between calcium, lipid, fibrous and thrombus components. The signal intensities on an MR image reflect the biochemical environment of protons in the tissue of interest, allowing tailoring of sequences to optimise the visualisation of specific tissue types. It is possible also to acquire three dimensional datasets that provide reproducible quantitative tissue information from isotropic voxels. MR can also provide contrast between the vessel wall and adjacent lumen by using flow-sensitive pulse sequences. Thus, MR can be used to image the vessel lumen in terms of flowing blood as well as simultaneously providing tissue information that describes the vessel wall (Fig. 1.5). The typical morphology of a vulnerable plaque consists of a thin fibrous cap which may be ulcerated or fissured, overlying a large necrotic core. The different properties of these components have been characterised in terms of their signal intensity characteristics on T1, T2, and intermediate-weighted images.

Fig. 1.5 Carotid MR angiogram (left) and cross sectional MR black blood images of carotid arteries (middle and right) (Fayad and Fuster 2001).



With the help of dedicated software programmes, it is possible to utilise the images provided to acquire accurate and reproducible measurements of plaque volume. Yuan *et al* assessed carotid wall area measurements in patients scheduled for carotid endarterectomy with high resolution MRI and found that results strongly agreed with ex-vivo measurements, with high within- and between-reader agreements (Yuan, Beach et al. 1998). Cai *et al* have demonstrated the ability of high-resolution multicontrast MRI to classify intermediate to advanced atherosclerotic lesions as described in the AHA classification (Cai, Hatsukami et al. 2002). Type III lesions, which consist of diffuse or eccentric thickening of the vessel wall containing lipid pools, appear as focal areas of high signal within the wall on T1-weighted and PD images. Type IV or V lesions, containing lipid or necrotic core, may show high signal intensity or iso-signal intensity on

T1-weighted, high or iso-signal intensity on PD weighted, and varied signal intensity on T2-weighted images (Cai, Hatsukami et al. 2002). Lumen surface defect and recent haemorrhage are detectable by multicontrast MR (Yuan, Mitsumori et al. 2001; Yuan, Zhang et al. 2002). Intact fibrous cap is seen as a band of low signal on TOF images, with the absence of this band indicating thin or absent cap. Plaque rupture is suspected when this high signal band is absent on TOF and a region of high signal intensity is seen adjacent to the lumen. Recent haemorrhage is seen as high signal intensity in both T1 weighted and TOF images. The calcification seen in type VII plaque is detectable by the presence of irregular low signal intensity on all contrast-weighted images. Type VIII plaque is highly fibrotic, with little or no lipid core, and is more a diagnosis of exclusion when a stenosis is seen without the characteristics which classify plaques as above.

Digital subtraction MR angiography is currently used in clinical practice in order to assess vessel stenosis. However, the information provided from high resolution magnetic resonance imaging with dedicated surface coils promises advancing imaging of both the carotid artery lumen as well as plaque morphology. This will greatly improve our ability to identify high risk patients. In addition, it will improve our understanding of plaque development, behaviour and its acute consequences as well as aiding in the development and targeting of novel therapeutic strategies to prevent progression to cerebral infarction.

1.6 AIMS

1.6.1 Chapter 3

The long term consequences of vascular disease require the provision of substantial health care resources. The identification of high risk patients is essential for appropriate and efficient treatment. Screening tools must be able to produce reliable and reproducible data. We aim to establish that, using multidetector computed tomography, CAC score can be measured in an acceptably reproducible manner.

1.6.2 Chapter 4

Aggressive lipid lowering has a major role in the treatment of established atherosclerotic disease. The presence of coronary artery calcification is a surrogate for the degree of plaque burden. We aim to establish the effect of lipid lowering therapy on the progression of coronary calcification as assessed by computed tomography.

1.6.3 Chapter 5

Coronary artery bypass graft stenosis and occlusion may occur in the early post-operative period or as a late complication. The assessment of graft patency in a non-invasive and readily applicable manner would have major benefits for the management and treatment of patients with prior CABG. We aim to determine whether MDCT can be used reliably to predict graft patency in patients who have undergone coronary artery bypass grafting.

1.6.4 Chapter 6

The identification of high risk patients in a safe, non-invasive and timely manner is essential for appropriate and efficient treatment. Understanding the mechanisms of plaque remodelling also has the potential to develop novel therapeutic strategies to aid in treatment and primary prevention of cardiovascular diseases. We will investigate the role of magnetic resonance imaging techniques to assess plaque volume and detect the characteristics associated with active inflammation following acute carotid plaque rupture.

1.7 HYPOTHESES

Non-invasive cardiac imaging modalities will be employed to test the following hypotheses:

- Computed tomography CAC scoring is reproducible.
- Statin therapy reduces the progression of CAC.
- Computed tomography angiography can be used reliably to determine coronary artery bypass graft patency.
- MRI can be employed in a timely and reproducible manner to assess plaque volume and detect characteristics associated with active inflammation following acute carotid plaque rupture.

CHAPTER 2

METHODOLOGY:

COMPUTED TOMOGRAPHY CORONARY CALCIUM SCORING, COMPUTED TOMOGRAPHY ANGIOGRAPHY AND MAGNETIC RESONANCE ANGIOGRAPHY

2.1 INTRODUCTION

The long term consequences of vascular disease require the provision of substantial health care resources. With recent advances in computer assisted imaging technology, it has become possible to perform a wide range of medical imaging non-invasively. This has the potential both in terms of screening for the presence of vascular disease, and in terms of diagnosis of established disease in order to target treatment and secondary prevention. In this thesis, two principle imaging modalities have been employed: computed tomography and magnetic resonance imaging.

2.2 ETHICAL CONSIDERATIONS

All studies were undertaken in accordance with the Declaration of Helsinki of the World Medical Association and with the approval of the local Research Ethics Committee. The written informed consent of each subject or patient was obtained before entry into the study.

Ethical approval had been applied for and granted prior to my involvement in the SALTIRE study (Chapters 3 and 4)). For the work undertaken in Chapters 5 and 6, I submitted applications to the Local Research and Ethics Committee (LREC) which included drafting of patient and medical practitioners information summary, invitation leaflets , protocol summaries and consent forms as well as attendance at the committee meeting. Applications were also submitted to the hospital Reasearch and Development (R&D) and Clinical Research Facility (CRF) departments before the studies could commence.

2.3 PATIENT

2.3.1 Effect Of Statin Therapy On Coronary Artery Calcium Score

The study population uses a sub-group of the Scottish Aortic stenosis Lipid-lowering Therapy, Impact on REgression (SALTIRE) trial which assessed the effect of aggressive lipid-lowering therapy in patients with calcific aortic stenosis (Cowell, Newby et al. 2005). Patients aged > 18 years, with calcific aortic stenosis (grade 1-3 calcification on echocardiography) and a peak post-valve velocity of ≥2.5 m/s were recruited from eight hospital centres across the South East of Scotland. Exclusion criteria were women of child-bearing potential without contraception, active or chronic liver disease, history of alcohol or drug misuse, severe mitral stenosis (valve area <1 cm^2), severe mitral or aortic regurgitation, marked left ventricular dysfunction (ejection fraction <35%), planned aortic valve replacement, intolerance to statins, patients who were taking or would in the opinion of the treating physician benefit from statin therapy, baseline serum total cholesterol of <4.0 mmol/L, and permanent pacemaker or cardiodefibrillator. For the purposes of the substudy, we also excluded patients who had no coronary artery calcification on computed tomography.

Between March 2001 and April 2002, the blinded study co-ordinator randomised eligible patients by the minimisation technique using a dedicated locked computer program (Edinburgh University) which incorporated eight baseline variables: age, sex, smoking habit, hypertension, diabetes mellitus, serum cholesterol concentration, peak aortic jet velocity and aortic calcium score. Patients were assigned either to atorvastatin 80 mg

daily or matched placebo (Pfizer Ltd., Tadworth, UK) as a single daily dose using numbered containers.

Patients were assessed at baseline, 2 months, 6 months and every 6 months thereafter for a minimum of 2 years. Clinical evaluation included assessment of functional status, adverse events and biochemical blood analysis. Serum hsCRP concentrations were determined using a highly sensitive immunonephelometric method (Dade Behring Ltd, Milton Keynes, UK) as previously described. All patients underwent computed tomography within the month before randomisation to study therapy and at each annual visit. Randomised patients who were subsequently commenced on open label statin therapy by their attending physician were immediately scanned and withdrawn from further observation.

2.3.2 CT Coronary Angiography In Detection Of Coronary Artery Bypass Graft Patency

Patients who were scheduled for routine coronary angioplasty in the Department of Cardiology, Royal Infirmary of Edinburgh, were approached prior to their procedure and offered an information leaflet along with an informal discussion regarding the rationale behind the study, and what their involvement would entail. Patients were invited to join the study and followed up by letter and telephone call following their procedure. If they agreed to participate in the study, a date for CTA was agreed. Three dedicated CT appointments were reserved for study participants in the Department of Radiology, Royal Infirmary of Edinburgh, each week.

Fifty consecutive patients who had undergone previous coronary artery bypass graft surgery and were listed for diagnostic coronary angiography between June 2004 and June 2005 were recruited into the study. Exclusion criteria were the presence of implanted metallic cardiac devices which may interfere with image quality (prosthetic heart valves, implantable pacemaker or cardiodefibrillator), renal impairment, atrial fibrillation or those patients unable to tolerate the supine position. The study was conducted with the approval of the local research ethics committee, in accordance with the Declaration of Helsinki and the written informed consent of each subject.

Coronary artery bypass graft patency was documented at the time of coronary angiography. Computed tomography was performed, where possible, within one month of coronary angiography.

2.3.3 Novel Magnetic Resonance Imaging Techniques And Remodelling Of Complex Carotid Atherosclerosis

Acute Neurovascular clinics take place twice weekly in the Western General Hospital, Edinburgh. I, assisted by my colleague Miss A Burdess, attended these clinics each week and sat in on the consultation with the Consultant Stroke Physician. Following patient consultation, information leaflet was provided and, if the patient was interested in participating in the study, they were accompanied to their Duplex ultrasound appointment in order to ascertain if they met the criteria outlined below. Following this, I scheduled

an appointment for the patient to attend for MRI scan, ideally within one week, by liasing with the research dedicated MRI scanning staff at the Western General Hospital.

Twelve consecutive patients attending the acute neurovascular clinics at the Western General Hospital in Edinburgh were recruited in to the study. Inclusion criteria were symptoms and signs of an acute transient ischaemic attack, amaurosis fugax or stroke within 7-14 days of symptom onset and Doppler ultrasound documentation of ipsilateral complex carotid plaque of >40% luminal stenosis to account for their clinical symptoms and presentation. Exclusion criteria were women of child bearing potential, renal or hepatic failure, severe or significant co-morbidity or being unable to tolerate the supine position.

Magnetic resonance imaging was performed within four weeks of symptom onset. In order to assess reproducibility, the scan was repeated within one week.

2.4 MDCT CORONARY ARTERY CALCIUM SCORING

2.4.1 Computed Tomography

Computed tomography was performed by a single blinded operator using a double-helix scanner (Twin II Flash; Philips Medical Systems (UK) Limited, Stevenage, UK) and calibrated against a standard phantom. Images were acquired in 2.7-mm slices (with a 0.75 s full 360° scan mode) through the region of the coronary arteries with a pitch of 0.7 and an increment of 1.3 mm during held inspiration. Exposure factors were 120 kV at 270 mAs and the scan angle was 360°.

Off-line analyses were conducted using an automated, computerised software program (Picker Cardiac Scoring). This employs a Agatston scoring method, producing sensitivity and specificity comparable to electron beam computed tomography. Each image was evaluated consecutively, with the threshold for the presence of calcium set at 130 Hounsfield units (HU) with an area of greater than or equal to 1 mm^2. For each grouping of pixels with an area greater than 1mm and a peak intensity greater than 130 HU, the maximum attenuation was recorded. The Agatston score of each lesion was then calculated as the product of its area and a weighting factor based on its maximum attenuation (Table 2.1). A total coronary calcium score was determined by summing individual lesion scores for each of three anatomic sites (left anterior descending, circumflex and right coronary arteries) for each slice. The coronary artery calcium score has subsequently been divided into five categories for stratification of risk for a subsequent cardiac event (Table 2.2) (Rumberger, Brundage et al. 1999).

I performed the CAC scoring for all patients on the Philips workstation in the Borders General Hospital outside of normal working hours, when the scanner was not in routine clinical use. I was trained in the technique by Dr John Reid, Consultant Radiologist. The technique involves drawing an individual region of interest around the calcified areas (>130HU) for each of the three vessels, on each CT image, thereby acquiring an individual vessel score and a total calcium score for each patient on each visit.

Table 2.1 CT attenuation weighting factors

Weighting factor	Hounsfield Unit range
1	130 – 199
2	200 – 299
3	300 – 399
4	>400

Table 2.2 CAC score – risk stratification

CAC score	Risk for a subsequent cardiac event
0	nil
1 - 10	Minimal
11 – 100	Mild
101 – 400	Moderate
>400	Severe

Scans were scored using both the Agatston (130 HU threshold) and the modified Agatston (90 HU threshold) methods. The former has been shown to reduce interobserver and interscan variation compared to the threshold of 90 HU. To assess the reproducibility of the method, repeated baseline computed tomography scans were performed within 4 weeks of each other in an unselected random sample of 16 patients.

2.4.2 Reproducibility of MDCT Calcium Scoring

To assess the reproducibility of the method, repeated baseline computed tomography scans were performed within 4 weeks of each other in a random sample of 16 patients.

2.5 COMPUTED TOMOGRAPHY ANGIOGRAPHY

2.5.1 Coronary Angiogram

Coronary angiography was performed by an experienced cardiologist via standard percutaneous approach, using 6 French Judkins catheters. Images were obtained on a Innova digital flat plate system (Advantx, GE Medical Systems) following i.v. bolus injection of Niopam contrast agent (Bracco, Bucks, UK). Selective catheterisation of grafts or graft stumps was performed.

2.5.2 MDCT Angiogram

I supervised all patient studies, which involved meeting the patients in the Radiology Departmens prior to the study in order to ensure they understood the procedure and to obtain consent. I supervised the scans themselves, including gaining intravenous access and monitoring the patients following the scan to ensure that there was no sign of contrast reaction.

MDCT angiography was performed using a 16-slice CT scanner (Aquilon; Toshiba, Tustin, CA). Prior to scanning, a 20- or 18- guage iv cannula was sited in the right arm. The patient was placed in a supine position in the gantry and the arms placed above the

patient's head with the ECG leads out of the scan range. The scan volume was defined based on expected location of the coronary arteries and grafts, following a scout view. In patients with known internal mammary artery grafts, this area was extended to the origin of the IMA at the proximal subclavian arteries. Fixed scanning variables included a gantry rotation time of 0.5 seconds, 16 x 1 mm detector collimation, 0.35 to 0.5 x 0.35 to 0.5 mm pixel size, 135kV, 250 to 300 mA, 0.25 pitch, and inspiratory breath hold time 20-30 seconds. Iomeprol, 100 mL (400-strength, Bracco, Bucks, UK) contrast agent was administered intravenously. The ^{SURE}Cardio acquisition feature was utilised, which monitors the patient's heart rate for five consecutive beats, calculates an average and automatically selects optimal scan parameters. The contrast bolus was monitored using the ^{SURE}Start feature to initiate imaging when contrast density in the ascending aorta is 160-180 HU. The images were obtained during inspiratory breath hold and retrospectively cardiac gated. The reconstruction set was limited to one phase at 75% of the R-R interval. From these images, one slice was selected to demonstrate best the three main coronary arteries at the mid-heart level. The selected slice was then reconstructed for the entire cardiac cycle at 20 ms intervals. From these images, the phase which best demonstrated the coronary arteries at this slice position were selected and the entire volume reconstructed at the selected phase (Figure I). The images were transferred to a dedicated workstation (Vitrea v3.5; Vital Images, Plymouth, MN).

2.5.3 Data Analysis

Two-dimensional axial, multiplanar reconstruction and three-dimensional reconstructions with volume rendering techniques were analysed by a radiologist who was familiar with

the cardiac anatomy but blinded to the result of the coronary angiogram. Image quality was graded in terms of eligible or insufficient (motion artefact, artefact caused by surgical clip) and eligible grafts were assessed in terms of patency and the presence and location of significant stenoses (stenosis >50% luminal diameter) were noted. The results of MDCT were compared with coronary angiography, the gold standard reference. Radiation dose for invasive coronary angiography and MDCT angiography was calculated from the documented dose area product ($cGycm^2$) and dose length product (mGycm) respectively.

2.6 MAGNETIC RESONANCE ANGIOGRAPHY

2.6.1 Imaging protocol

Immediately prior to the first scan appointment, I met with all patients to go over the study and answer any questions as well as to obtain consent. I also filled in a checklist for each patient to ensure they had no metallic devices or contraindications to MR scanning. I supervised all scans including obtaining intravenous access, administering the contrast ageny and monitoring patients afterwards for any signs of contrast reaction.

In order to assess feasibility, each subject underwent the following magnetic resonance imaging protocol. Subjects were placed in a head collar to reduce movement artefact. Carotid surface coils (bilateral four channel phased array) were applied to maximise resolution, and scanning was cardiac synchronised to minimise pulsation artefacts. A research dedicated magnetic resonance imaging scanner (1.5T GE Echospeed) performed

a two dimensional time of flight (2D TOF) localiser view with a field of view FOV of 22 cm. The carotid bifurcation was identified from the localiser sequence with subsequent sequences, based around the carotid bifurcation, consisting of axial T1 DIR 'black blood' with a field of view (FOV) of 14 cm; proton density and T2 fast spin echo (FSE) with FOV of 13 cm; and 3D TOF with FOV of 13 cm before and after enhancement with an intravenous bolus of 0.1 mmol/kg Dotarem® (Gadoterate meglumine, Guerbet S.A., Paris, France). For each sequence this resulted in 9 slices, each 2mm thick, centred upon the carotid bifurcation of interest, thus giving coverage of 1.8 cm. In order to assess reproducibility, the imaging protocol was then repeated within 2 weeks of the first assessment. Measurements of plaque area and volume were then compared between the two sets of scans.

In order to assess neovascularisation, four subjects underwent an initial DIR axial scan (11 slices, 2mm thick), followed by a fast spoiled gradient recalled echo sequence (FSPGR), with T-1 weighted images of the diseased carotid artery at 9 locations, centred on the carotid bifurcation. Following the method of Kerwin *et al*, 2003, each acquisition was repeated 10 times, with a repetition interval of 16 seconds. Coincident with the second image in the sequence, 0.1 mmol/kg of Dotarem® was injected at a rate of 2mLs/s via a power injector.

2.6.2 Quantification of Carotid MR images

Through collaboration with the Image Analysis Core of the Wellcome Trust Clinical Research Facility, digital images were quantitatively assessed using the development of

existing software. Using this software, the cross-sectional lumen and outer vessel wall boundaries were manually traced and cross-sectional areas calculated. Plaque area was defined as the vessel area minus the lumen area. Overall plaque area and volume were measured with reference to the carotid bifurcation to ensure consistency and comparability of study segments. Variation in pixel intensities within the plaque was also assessed. All scans, prior to measurements, were set at the same intensity level (Level 1111, Width 222). The software package was then used to calculate the occurrences of different intensity levels within the plaque. Histograms of occurrences against intensity were then generated, with the hypothesis that heterogeneous plaques would yield differing histograms to homogeneous plaques.

Data for each patient was assessed by an independent experienced neuroradiologist with respect to regional plaque densities using existing criteria for lipid and fibrous plaques (Table 2.3). An overall Modified AHA Classification for MRI was allocated to each subject. This was then compared with the overall conventional histological AHA classification for those subjects who had undergone endarterctomy.

Table 2.3 Conventional and Modified AHA Classification of Atherosclerotic Plaque

Conventional AHA Classification	**Modified AHA Classification for MRI**
Type I: initial lesion with foam cells	Type I–II: near-normal wall thickness, no calcification
Type II: fatty streak with multiple foam cell layers	
Type III: preatheroma with extracellular lipid pools	Type III: diffuse intimal thickening or small eccentric plaque with no calcification
Type IV: atheroma with a confluent extracellular lipid core	Type IV–V: plaque with a lipid or necrotic core surrounded by fibrous tissue with possible calcification
Type V: fibroatheroma	
Type VI: complex plaque with possible surface defect, hemorrhage, or thrombus	Type VI: complex plaque with possible surface defect, hemorrhage, or thrombus
Type VII: calcified plaque	Type VII: calcified plaque
Type VIII: fibrotic plaque without lipid core	Type VIII: fibrotic plaque without lipid core and with possible small calcifications

Finally, neovascularisation was determined by the degree of gadolinium enhancement of the plaque. After the acquisition of the nine locations around the carotid bifurcation, the area of the plaque on each location was manually traced as described above. These regions of interest (plaque areas) were then aligned for each of the 11 time points and analysed for changes in signal intensity over time.

2.7 STATISTICS

2.7.1 Reproducibility of MDCT Coronary Calcium Scoring

To assess the reproducibility of the method, repeated baseline CT scans were performed within four weeks of each other in a random sample of 16 patients.

To determine the interscan correlation between calcium scores, the data were log transformed to reduce skew, and assessed using the method of Bland and Altman (Bland and Altman 1986). Bland Altman plots of the difference between the log total calcium score for each scan against the average between the two measurements were used to show the relationship between variability and calcium distribution. Coefficient of variation for coronary artery calcium score as measured by MDCT was calculated as the standard deviation divided by the mean. This is expressed as a percentage and allows comparison with reproducibility data in published literature.

Analysis of the effect of atorvastatin on the coronary calcium score was performed in collaboration with the Medical Statistics Department of the University of Edinburgh. Random coefficients models were used as the data had a hierarchical (or multilevel) structure. In the simplest regression problem, one static observation per covariate is collected, or multiple observations for just one subject. However, in hierarchical models such as we have, data are structured across multiple levels, i.e. multiple observations for multiple subjects so there will be some variability within each subject (within-subject variance) and some variability between subjects (between-subject variance). In these models, the variability at each level is distinct and needs to be accounted for separately at

each level in whatever analysis is undertaken. The random coefficients model is designed for doing just this whilst accounting for these multiple sources of variation and prevents a falsely statistically significant difference from being obtained.

CAC scores are expressed in arbitrary units (AU) using the 130HU threshold. The calcium scores and hsCRP concentrations were not normally distributed and the data are presented as median with interquartile ranges or mean and standard deviation following logarithmic transformation (log AU). The primary end-point, the rate of change of coronary calcium scores, was analysed using random coefficients models, for the reasons above, after logarithmic transformation of the scores. In summarising the data, the change in coronary artery calcium scores was calculated by dividing the change between the baseline and final scores by the duration of follow-up. Rate of change in coronary calcium score is expressed as percentage change per year or as absolute change in the logarithm of the coronary artery calcium score.

2.6.2 CT Coronary Angiography in Detection of Coronary Artery Bypass Graft Patency

The results of MDCT were compared with coronary angiography, the gold standard reference. Sensitivity, specificity, positive and negative predictive value (PPV and NPV respectively) was calculated for the detection of graft patency by MDCT. Sensitivity was calculated as true patent/(true patent + false occluded) grafts. Specificity was calculated as the number of true occluded/(true occluded + false patent) grafts. The positive predictive value was a result of true patent/(true patent + false patent), and the negative

predictive value as true occluded/(true occluded + false occluded). The accuracy was determined by (true patent + true occluded)/total number of grafts. 95% confidence intervals were calculated by multiplying the standard error by 1.96.

2.6.3 Novel Magnetic Resonance Imaging Techniques and Remodelling of Complex Carotid Atherosclerosis

This was a preliminary study which aimed to establish the feasibility and reproducibility of novel magnetic resonance based techniques. An accurate estimate of statistical power was not possible. As a pilot study the data generated will permit a formal power calculation to be made for potential future clinical trials. For the parametric and volumetric variables described above, reproducibility was assessed by the method of Bland and Altman (Bland and Altman 1986), whereby plots of the difference between measurements against the average measurements were performed. The method of measurement was judged as being repeatable if 95% of the differences were of less than two standard deviations.

CHAPTER 3

REPRODUCIBILITY OF THE CT CORONARY CALCIUM SCORE

3.1 ABSTRACT

Introduction Coronary artery calcification is virtually pathognomonic of atherosclerosis, and can be quantified in a non-invasive fashion using computed tomography. In order to be used reliably, measurements obtained should be reproducible and the interscan variability low.

Methods In order to assess the reproducibility of the method, repeated baseline computed tomography scans were performed within 4 weeks of each other in an unselected random sample of 16 patients who took part in the Scottish Aortic Stenosis Lipid lowering Therapy, Impact on Regression trial. Coefficients of variation were calculated.

Results Overall, the differences on the log scale correspond to a coefficient of variation of 28% for both variables, but when restricted to the ten pairs with a geometric mean score above 100, the coefficient of variation was 10% for both variables.

Conclusion We have demonstrated good reproducibility of coronary artery calcification scores in patients with scores of greater than 100 AU.

3.2 INTRODUCTION

Pathological, angiographic and fluoroscopic studies have shown that coronary artery calcification is virtually pathognomic of atherosclerosis (Frink, Achor et al. 1970; Rifkin, Parisi et al. 1979). CT is a well validated means of calculating the coronary artery calcium score, with a high sensitivity and negative predictive value when compared to fluoroscopy (Agatston, Janowitz et al. 1990). Coronary artery calcium score measurements have been used to stratify individual cardiac risk, to determine the presence of coronary disease and to monitor disease progression and response to therapy (Detrano, Doherty et al. 2000; Moser, O'Keefe et al. 2003; Pletcher, Tice et al. 2004). In order to monitor the progression of disease in a reliable fashion, the measurements obtained should be reproducible and the interscan variability low. Research, however, has cast doubts on the reliability of MDCT (Van Hoe, De Meerleer et al. 2003). Studies have highlighted the importance of the choice of timing during the cardiac cycle on the reconstruction window. One such study found that spiral CT enables reproducible quantification of coronary artery calcium in retrospectively ECG-gated images (Ohnesorge, Flohr et al. 2002), findings which have since been corroborated in other studies (Kopp, Ohnesorge et al. 2002).

The Scottish Aortic Stenosis Lipid lowering Therapy, Impact on REgression (SALTIRE) trial was a prospective double blind randomised controlled study of intensive lipid lowering therapy in patients with calcific aortic stenosis (Cowell, Newby et al. 2005). As part of this trial, aortic valve and coronary artery calcium scores are measured using

helical computed tomography. The aim of this substudy was to assess reproducibility of the coronary artery calcium score.

3.3 METHODS

The patient population and study protocol was as for Chapter 4, *Progressive coronary calcification despite intensive lipid-lowering therapy: a randomised controlled trial.* In order to assess the reproducibility of the method, repeated baseline computed tomography scans were performed within 4 weeks of each other in a random sample of 16 patients.

To determine the interscan correlation between calcium scores, the data were log transformed to reduce skew, and assessed using the method of Bland and Altman (Bland and Altman 1986). Bland Altman plots of the difference between the log total calcium score for each scan against the average between the two measurements were used to show the relationship between variability and calcium distribution. Coefficient of variation for coronary artery calcium score as measured by MDCT was calculated as the standard deviation divided by the mean, expressed as percentage.

3.4 RESULTS

The reproducibility of the LAD coronary score and of the total coronary score was examined using the approach of Bland and Altman (Bland and Altman 1986). Without transformation, the difference between replicate observations tended to increase with the magnitude of the measurement. After logarithmic transformation, higher values showed stable differences, but differences were higher at the lowest scores (Figs. 3.1 and 3.2). Overall, the differences on the log scale correspond to a coefficient of variation of 28% for both variables, but when restricted to the ten pairs with a geometric mean score above 100, the coefficient of variation was 10% for both variables.

Figs 3.1 and 3.2.

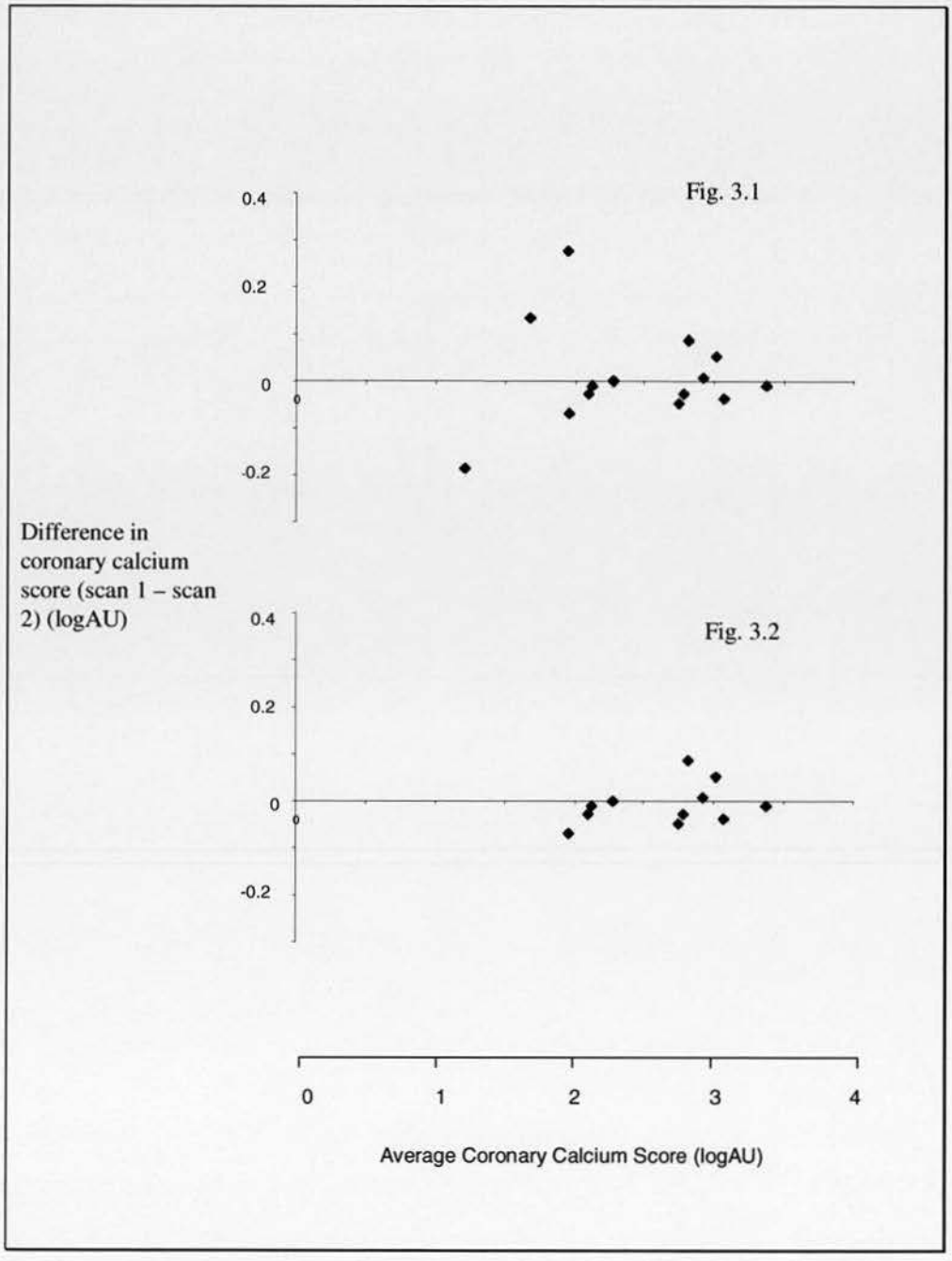


Fig. 3.1 Bland Altman plot showing all reproducibility datasets.

Fig. 3.2 Bland Altman plot showing reproducibility data for patients with a total CAC score of >100 AU.

3.5 DISCUSSION

Histopathologic studies have demonstrated the correlation of coronary artery calcification with the presence of atherosclerosis (Rumberger, Simons et al. 1995). There appears to be a relationship between the extent of coronary calcification and the severity of luminal stenosis, as measured by invasive coronary angiography (Rumberger, Sheedy et al. 1997). However, angiographic severity of luminal stenosis does not predict risk of future cardiac events, and is therefore of less use as a screening tool (Ambrose, Tannenbaum et al. 1988). On the other hand, a high coronary artery calcium score has been shown to be an independent predictor of coronary heart disease events (Wayhs, Zelinger et al. 2002; Pletcher, Tice et al. 2004) and indeed progression of coronary artery calcification has been shown to be greater in patients with future myocardial infarction (Raggi, Cooil et al. 2003).

Controversy continues to exist over the role of coronary artery calcium screening and, as yet, there are no clear guidelines regarding specific indications. Its use has been suggested in screening of asymptomatic patients with other risk factors for cardiac disease (Moser, O'Keefe et al. 2003), in monitoring disease progression (Shemesh, Apter et al. 2000; Budoff and Raggi 2001) and also in monitoring disease response to therapy (Callister, Raggi et al. 1998; Budoff, Lane et al. 2000; Budoff and Raggi 2001; Achenbach, Ropers et al. 2002). The final point, namely monitoring disease response to therapy, is the most controversial of all and, until recently, the only published data were retrospective or observational studies. Recently, however, definitive prospective randomised studies have proved that statin therapy has no effect on the progression of

coronary artery calcification, thereby removing the indication for serial scanning as a method of measuring disease response to therapy (Arad, Spadaro et al. 2005; Raggi, Davidson et al. 2005). Nonetheless, whether the coronary artery calcium score is used as a one off screening modality for risk stratification, or as a way of monitoring disease progression, the importance of a reliable and reproducible method is paramount.

Many different methods of calculating agreement have been described. Our results demonstrate that the range of arithmetic differences between the two repeated measures increased in proportion to the increase in the mean score. As described by Bland and Altman, logarithmic transformation of the scores results in a uniform distribution of differences over the range of the mean. The mean and standard deviation of differences can then be calculated and a meaningful comparison of differences evaluated across a range of coronary artery calcium scores. After logarithmic transformation, higher values showed stable differences. Overall, the differences on the log scale correspond to a coefficient of variation of 28% for both variables, but when restricted to the ten pairs with a geometric mean score above 100, the coefficient of variation was 10% for both variables. A threshold of >100 has been shown to be highly sensitive for the prediction of future hard coronary events (Arad, Spadaro et al. 1996) therefore, importantly, we have demonstrated good reproducibility for scores which may be clinically significant. Similar results were achieved by Lu *et al*, who reported mean interscan variability of 21.5% for the Agatston scoring method. They also found that variability decreased with increasing coronary calcium score (34.6% for a score of 11-50 and 9.4% for a score of 400 – 1000) (Lu, Budoff et al. 2002). Budoff *et al* report an even greater spread of

variability for different CAC scores, with variability of 61.3% for scores of 1-30, dropping to 8.2% for scores of >400 (Budoff, Kessler et al. 2008). Different methods of quantifying calcification exist, and it has been suggested that volumetric scores may be more reproducible than the Agatston scoring method. However, a study of 6814 patients found that calcium volume scores were only slightly more reproducible than the Agatston scoring method (mean relative difference 18.3 vs 20.1 for volumetric and Agatston score respectively) (Detrano, Anderson et al. 2005).

Study Limitations

Whilst we found our interscan variability to be similar to published data, we did not investigate other means of variability. Importantly, the scans were interpreted by a single operator which is not feasible in regular clinical practice. An obvious source of variability is interobserver variability which we did not assess. In addition, intra-observer variability must be considered. In order to assess the variability of a scoring method more accurately, it would be helpful to measure these additional parameters.

3.6 CONCLUSION

We have demonstrated good reproducibility of coronary artery calcification scores in patients with scores of greater than 100 AU. A threshold of >100 has been shown to be highly sensitive for the prediction of future hard coronary events (Arad, Spadaro et al. 1996) therefore, importantly, we have demonstrated good reproducibility for scores which may be clinically significant.

CHAPTER 4

PROGRESSIVE CORONARY CALCIFICATION DESPITE INTENSIVE LIPID-LOWERING THERAPY: *A RANDOMISED CONTROLLED TRIAL*

Houslay ES, Cowell SJ, Prescott RJ, Reid J, Burton J, Northridge DB, Boon NA, Newby DE.

Progressive coronary calcification despite intensive lipid-lowering treatment: a randomised controlled trial. *Heart* 2006 Sep;**92**(9):1207-12.

http://heart.bmj.com/cgi/content/full/92/9/1207

4.1 ABSTRACT

Objectives Observational studies have suggested that statin therapy may induce regression of coronary artery calcification. In a substudy of a trial recruiting patients with calcific aortic stenosis, we evaluated the effect of intensive lipid-lowering therapy on coronary artery calcification.

Methods In a double blind randomised controlled trial, 102 patients with calcific aortic stenosis and coronary artery calcification were randomised using the minimisation technique to atorvastatin 80 mg daily or matched placebo. Coronary artery calcification was assessed annually by helical computed tomography.

Results Forty-eight patients were randomised to atorvastatin and 54 to placebo with a median follow-up of 24 months (interquartile range, 24-30). Baseline characteristics and coronary artery calcium scores were similar in both groups. Atorvastatin therapy reduced serum low-density lipoprotein cholesterol (-53%; P<0.001) and C-reactive protein (-49%; P<0.001) concentrations whilst there was no change with placebo (-7% and +17%; P>0.95 for both). The rate of change in coronary artery calcification was 26%/yr (0.234 (SE 0.037) logAU/yr; n=39) in the atorvastatin group and 18%/yr (0.167 (SE 0.034) log AU/yr; n=49) in the placebo group: geometric mean difference of +7%/yr (95% confidence intervals -3% to +18%; P=0.18). There was no correlation between serum low-density lipoprotein concentrations and the rate of progression of coronary calcification (r=0.05, P=0.62).

Conclusion In contrast to previous observational studies, this randomised controlled trial has shown that, despite reducing systemic inflammation and halving serum low-

density lipoprotein cholesterol concentrations, statin therapy does not have a major effect on the rate of progression of coronary artery calcification.

4.2 INTRODUCTION

Coronary artery calcification is an independent risk factor for coronary heart disease with even low coronary calcium scores doubling the risk of coronary events (Pletcher, Tice et al. 2004). The relative risk associated with coronary calcification greater than that associated with established factors such as smoking, hypertension and diabetes mellitus. Progression of coronary artery calcification is associated with a higher incidence of coronary events even in those people who are asymptomatic at the time of initial scanning (Raggi, Cooil et al. 2003). Thus, the presence of coronary artery calcification is not only indicative of atheromatous plaque disease, but its progression may correspond with cardiovascular event rates.

Statin therapy has a proven role in the primary (Shepherd, Cobbe et al. 1995; Downs, Clearfield et al. 1998) and secondary prevention (1994; 1998; Lewis, Moye et al. 1998) of cardiovascular disease with incremental benefits seen with more intensive reductions in serum cholesterol concentrations (2002). Previous studies (Callister, Raggi et al. 1998; Achenbach, Ropers et al. 2002) have reported that statins can halt the progression and may even induce regression of coronary artery calcification. Indeed, the rate of progression of coronary artery calcification correlates with the average serum low-density lipoprotein (LDL) cholesterol concentration (Callister, Raggi et al. 1998). This has led to the use of computed tomography to monitor disease progression and response to treatment, particularly statin therapy. However, two recent trials have failed to demonstrate a benefit of statin therapy on the progression of coronary artery calcification in asymptomatic individuals (Arad, Spadaro et al. 2005; Raggi, Davidson et al. 2005).

The Scottish Aortic Stenosis Lipid lowering Therapy, Impact on REgression (SALTIRE) trial was a prospective double blind randomised controlled study of intensive lipid lowering therapy in patients with calcific aortic stenosis (Cowell, Newby et al. 2005). As part of this trial, aortic valve and coronary artery calcium scores are measured using helical computed tomography. The aim of this substudy was to assess the effect of atorvastatin 80 mg daily on the rate of progression of coronary artery calcification in patients with calcific aortic stenosis.

4.3 METHODS

4.3.1 Patient Population

Patients aged > 18 years, with calcific aortic stenosis (grade 1-3 calcification on echocardiography (Rosenhek, Binder et al. 2000)) and a peak post-valve velocity of ≥2.5 m/s were recruited from eight hospital centres across the South East of Scotland. Exclusion criteria were women of child-bearing potential without contraception, active or chronic liver disease, history of alcohol or drug misuse, severe mitral stenosis (valve area <1 cm^2), severe mitral or aortic regurgitation (Zoghbi, Enriquez-Sarano et al. 2003), marked left ventricular dysfunction (ejection fraction <35%), planned aortic valve replacement, intolerance to statins, patients who were taking or would in the opinion of the treating physician benefit from statin therapy, baseline serum total cholesterol of <4.0 mmol/L, and permanent pacemaker or cardiodefibrillator. For the purposes of the substudy, we also excluded patients who had no coronary artery calcification on computed tomography. The study was conducted with the approval of all the regional research ethics committee and in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject.

4.3.2 Study Protocol

Between March 2001 and April 2002, the blinded study co-ordinator randomised eligible patients by the minimisation technique (Treasure and MacRae 1998) using a dedicated locked computer program (Edinburgh University) which incorporated eight baseline variables: age, sex, smoking habit, hypertension, diabetes mellitus, serum cholesterol concentration, peak aortic jet velocity and aortic calcium score. Patients were assigned

either to atorvastatin 80 mg daily or matched placebo (Pfizer Ltd., Tadworth, UK) as a single daily dose using numbered containers.

Patients were assessed at baseline, 2 months, 6 months and every 6 months thereafter for a minimum of 2 years. Clinical evaluation included assessment of functional status, adverse events and biochemical blood analysis. Serum highly sensitive C-reactive protein (hsCRP) concentrations were determined using a highly sensitive immunonephelometric method (Dade Behring Ltd, Milton Keynes, UK). All patients underwent computed tomography within the month before randomisation to study therapy and at each annual visit. Randomised patients who were subsequently commenced on open label statin therapy by their attending physician were immediately scanned and withdrawn from further observation.

4.3.3 Computed Tomography

Computed tomography was performed by a single blinded operator using a double-helix scanner (Twin II Flash; Philips Medical Systems (UK) Limited, Stevenage, UK) and calibrated against a standard phantom. Images were acquired in 2.7-mm slices (with a 0.75 s full 360° scan mode) through the region of the coronary arteries with a pitch of 0.7 and an increment of 1.3 mm during held inspiration. Exposure factors were 120 kV at 270 mAs and the scan angle was 360°. Off-line analyses were conducted using an automated, computerised software program (Picker Cardiac Scoring). This employs a Agatston scoring method (Agatston, Janowitz et al. 1990), producing sensitivity and specificity comparable to electron beam computed tomography (Carr, Crouse et al. 2000).

Scans were scored using both the Agatston (130 HU threshold) and the modified Agatston (90 HU threshold) methods (Shemesh, Apter et al. 1995). The former has been shown to reduce interobserver and interscan variation compared to the threshold of 90 HU (Goldin, Yoon et al. 2001).

4.3.4 Data Analysis and Statistics

Coronary artery calcium scores are expressed in arbitrary units (AU) using the 130 HU threshold. The calcium scores and hsCRP concentrations were not normally distributed and data are presented as median with interquartile ranges or mean and standard deviation following logarithmic transformation (log AU). The primary end-point, the rate of change of coronary calcium scores, was analysed using random coefficient models (Brown and Prescott 1999) after logarithmic transformation of the scores. In summarising the data, the change in coronary artery calcium scores was calculated by dividing the change between the baseline and final scores by the duration of follow-up. Rate of change in coronary calcium score is expressed as percentage change per year or as absolute change in the logarithm of the coronary artery calcium score. As well as tests of significance, 95% confidence intervals are reported as appropriate. Statistical significance was taken as a two-sided P value <0.05.

4.4 RESULTS

Of 155 patients recruited into the SALTIRE trial, 102 had coronary calcification at baseline (Figure 4.1; CONSORT flow diagram of patients recruited into the trial and substudy) of whom 88 had at least two scans. Coronary calcification predominated in the left anterior descending artery (100% of patients) although it was also present in the circumflex (33%) and right (27%) coronary arteries. Baseline characteristics and coronary artery calcium scores were well matched in both treatment groups (Table 4.1) in the 88 evaluable subjects.

Figure 4.1

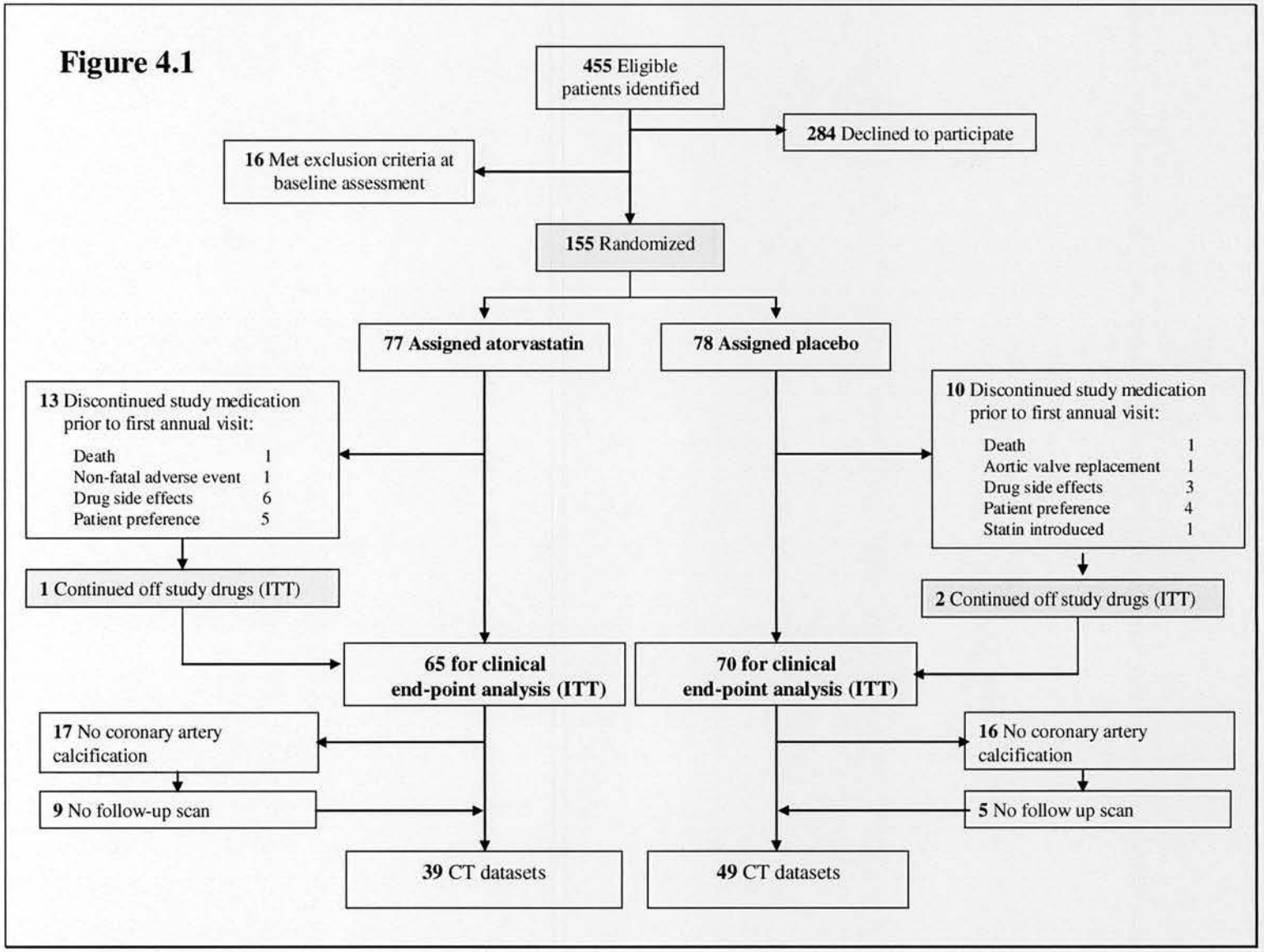

455 Eligible patients identified
284 Declined to participate
16 Met exclusion criteria at baseline assessment
155 Randomized
77 Assigned atorvastatin
78 Assigned placebo
13 Discontinued study medication prior to first annual visit:
Death 1
Non-fatal adverse event 1
Drug side effects 6
Patient preference 5
10 Discontinued study medication prior to first annual visit:
Death 1
Aortic valve replacement 1
Drug side effects 3
Patient preference 4
Statin introduced 1
1 Continued off study drugs (ITT)
2 Continued off study drugs (ITT)
65 for clinical end-point analysis (ITT)
70 for clinical end-point analysis (ITT)
17 No coronary artery calcification
16 No coronary artery calcification
9 No follow-up scan
5 No follow up scan
39 CT datasets
49 CT datasets

Table 4.1 Baseline subject characteristics

	Atorvastatin (n=39)	**Placebo** (n=49)
Age (years)	70 (8)	70 (9)
Sex (% male)	74	78
Body mass index	29 (5)	28 (5)
Cardiovascular risk factors		
Hypertension	22	28
Hyperlipidaemia	3	2
Diabetes Mellitus	0	2
Current smoker	5	10
Cardiovascular disease		
Coronary heart disease	7	13
Cerebrovascular disease	5	7
Peripheral vascular disease	3	7
Drug History		
Aspirin	17	26
ACE-inhibitor	7	8
Beta-blocker	11	15
Warfarin	4	8
Blood Pressure (mmHg)		
Systolic blood pressure	143 (18)	140 (19)
Diastolic blood pressure	82 (11)	78 (11)
Lipid Profile		
Total cholesterol (mmol/L)	5.7 (0.9)	5.5 (0.9)
LDL cholesterol (mmol/L)	3.6 (0.8)	3.4 (0.7)
HDL cholesterol (mmol/L)	1.5 (0.4)	1.5 (0.4)
Chol:HDL ratio	4.2 (1.2)	4.0 (1.0)
Triglycerides (mmol/L)	1.5 (0.8)	1.4 (0.7)
Coronary calcification score (AU)		
Left anterior descending	112 (40– 285)	207 (76 – 461)
Circumflex	0 (0 – 9)	0 (0 – 4)
Right	0 (0-29)	0 (0 – 0)
Total coronary score	195 (57 – 448)	235 (83 – 526)
Log total coronary score (log AU)	2.16 (0.68)	2.30 (0.65)

Continuous variables stated as mean (SD) or median (interquartile range).

Categorical variables stated as percent.

4.4.1 Effect of Atorvastatin Treatment

Patients were followed up for a median of 24 months (interquartile range, 24 to 30). Atorvastatin 80 mg daily more than halved serum LDL cholesterol concentrations (53 (SD 19) %; p<0.001), whilst placebo had no effect (Figure 4.2). This reduction in serum LDL cholesterol concentrations was associated with a marked decrease in serum C-reactive protein concentrations from 1.95 (interquartile range, 1.15 to 4.86) to 1.00 (0.49 to 2.31) mg/L (Wilcoxon Signed Rank, P<0.001; Figure 2). Atorvastatin was well tolerated with discontinuation of study medication in two patients on placebo and five patients on atorvastatin, predominantly as a result of gastrointestinal upset. One patient on atorvastatin had an increase in creatine kinase of >5-times the upper limit of normal without symptoms of myositis, and was withdrawn at the request of the Data Monitoring Committee. There were no cases of rhabdomyolysis.

4.4.2 Coronary Artery Calcium Score

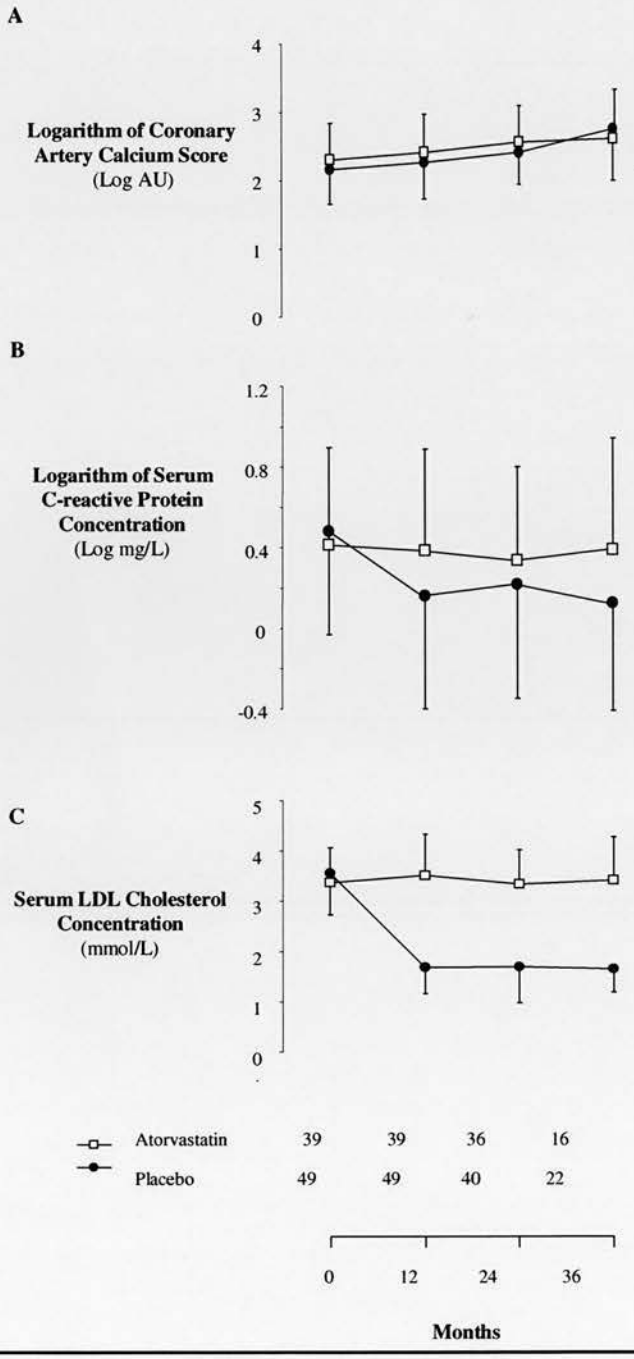
Atorvastatin did not affect the rate of progression of CAC score (Figure 4.2). Similar results were obtained when employing the 90 HU threshold (42 (SD 73) %/yr in the atorvastatin group and 29 (SD 37) %/yr in the placebo group; P=0.24). Serum LDL cholesterol concentrations did not correlate with the rate of progression of coronary artery calcification (r=0.05, P=0.62).

The primary analysis of the rates of change of coronary artery calcium scores was conducted on the logarithms of the scores using random coefficients models (Brown and Prescott 1999). This showed no difference between the average rates of change in the

two treatment arms (P=0.18). The mean coronary calcium score increased by 0.234 (SE 0.037) log AU/yr in the atorvastatin group and 0.167 (SE 0.034) log AU/yr in the placebo group. These figures correspond to a 26 %/yr increase in the atorvastatin group and 18 %/yr in the placebo group. The geometric mean (adjusted for baseline) is 7% higher at one year on atorvastatin compared to placebo, with 95% confidence limits ranging from 3% lower to 18% higher. The observed annual changes in CAC scores, calculated from the first to the last visit, are summarised in Figure 4.3.

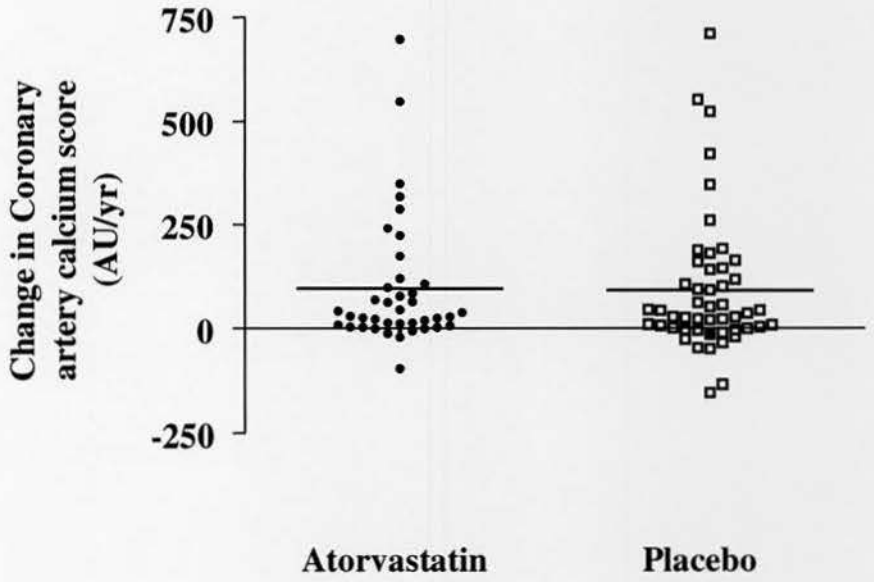
As anticipated in such a modest clinical trial, there were no significant differences in all-cause mortality, cardiovascular mortality or cardiovascular hospitalisation between the two groups.

Figure 4.2



Progression of (A) coronary artery calcification, (B) serum C-reactive protein concentrations ($P<0.001$, atorvastatin versus placebo), and (C) serum low-density lipoprotein (LDL) cholesterol concentrations ($P<0.001$, atorvastatin versus placebo) in patients treated with atorvastatin 80 mg daily (solid circles) or matched placebo (open squares).

Figure 4.3



Absolute rate of change in CAC score (AU per year for patients treated with atorvastatin 80 mg (solid circles) or matched placebo (open squares)).

4.5 DISCUSSION

We have confirmed that, despite marked reductions in serum LDL cholesterol and C-reactive protein concentrations, atorvastatin 80 mg daily did not halt the progression, or induce regression, of coronary artery calcification in patients with calcific aortic stenosis. Consistent with recent trials of asymptomatic individuals (Arad, Spadaro et al. 2005; Raggi, Davidson et al. 2005), our findings are in marked contrast to previous observational studies and suggest that the potential beneficial effects on coronary artery calcification have been over estimated.

Previous observational and non-randomised prospective studies (Callister, Raggi et al. 1998; Achenbach, Ropers et al. 2002) have suggested that reductions in serum LDL cholesterol concentrations decrease the progression of coronary calcification. However, not all observational studies have demonstrated consistent findings. In the largest observational study of 182 patients, Hecht and colleagues recently found no difference in the progression of coronary calcium scores in patients who were maintained on lipid lowering therapy and achieved significant reductions in serum LDL cholesterol concentrations (Hecht and Harman 2003). Observational data may be misleading and prospective randomised controlled trials are necessary to confirm or to refute these interesting preliminary observations. The recent BELLES trial (Raggi, Davidson et al. 2005) found no differential effect of pravastatin (40 mg daily) and atorvstatin (80 mg daily) on the progression of coronary artery calcification in 615 hyperlipidaemic post-menopausal women. However, study follow-up was brief (1 year) and there was no placebo control group. The St Francis Heart Study (Arad, Spadaro et al. 2005)

randomised 1,005 asymptomatic middle-aged men and women with high coronary artery calcium scores to combination atorvastatin 20 mg, vitamin C 1 g and vitamin E (alpha tocopherol) 1000 U daily or matching placebos. After 4.3 years of follow-up, there were no differences in the rate of progression of coronary artery calcification.

We have conducted a double blind randomised controlled trial using helical computed tomography in patients with aortic stenosis. Minimisation technique ensured good matching of the baseline characteristics of the patient population and reproducibility studies confirmed the validity of our repeated assessments. Although documenting very similar rates of progression of coronary calcification to previous studies (Callister, Raggi et al. 1998; Achenbach, Ropers et al. 2002; Hecht and Harman 2003), we have not observed a reduction in coronary calcification with intensive lipid lowering therapy despite more than halving serum LDL cholesterol concentrations.

Statin therapy has been extremely successful in the primary and secondary prevention of cardiovascular disease. Why then have we and others not observed a beneficial effect of statin therapy on coronary artery calcification? Unstable atherosclerotic plaques have a large lipid rich core, a preponderance of macrophages and foam cells, and a thin fibrous cap containing few smooth muscle cells (Davies 1997). It has been suggested that calcified lesions may be relatively more stable (Mintz, Popma et al. 1995), indicating a possible protective role of calcification in coronary plaques. Statin therapy produces many of its beneficial effects through plaque stabilisation. In both primate (Stary 2001) and swine (Daoud, Jarmolych et al. 1981) models, anti-atherosclerotic interventions are

associated with an increase in vascular fibrous tissue and calcification. This calcium deposition continues during the initial phase of plaque regression due to the death of foam cells and an increase in necrotic tissue. Thus vascular calcification may play a role in the initial stabilisation of atherosclerotic plaques. This is consistent with our findings and would account for the lack of effect on the progression of coronary artery calcification despite a reduction in serum C-reactive protein concentrations.

After the initial stabilisation of the atherosclerotic plaque, it would be anticipated that subsequent progression of coronary calcification would be inhibited. The present study was brief, and follow up was only continued for a median of 2 years. It would be important to extend our observations to 5 or more years to assess properly the impact of statin therapy on the long-term progression of coronary artery calcification. However, it should be acknowledged that the clinical benefits of statin therapy are apparent within the first few years (1998; Lewis, Moye et al. 1998; 2002), and in some cases the first few months (Schwartz, Oliver et al. 1998), of therapy. Moreover, the St Francis Heart Study demonstrated no beneficial effects despite 4.3 years of follow-up (Arad, Spadaro et al. 2005).

On the basis of previous non-randomised studies (Achenbach, Ropers et al. 2002), the practice of performing serial CT scans to monitor disease progression and the response to treatment has become widespread, especially in the North America. Our data, and that of the St Francis Heart Study (Arad, Spadaro et al. 2005) and the BELLES study (Raggi, Davidson et al. 2005), indicate that repeated scanning to assess response to statin therapy

is not justified. Indeed, the radiation dose incurred for such serial scans poses potential health risks, particularly when employing multidetector computed tomography scanners.

4.5.1 Study Limitations

There are several factors that should be taken into account when considering the results of our study. This was a substudy of the SALTIRE trial (Cowell, Newby et al. 2005) that recruited only patients with calcific aortic stenosis. However, our findings are consistent with two recent randomised controlled trials in asymptomatic younger individuals without valvular heart disease (Arad, Spadaro et al. 2005; Raggi, Davidson et al. 2005). Our study therefore suggests that failure of statins to restrict the progression of coronary artery calcification can be extended to include patients with valvular heart disease as well as more elderly populations. Moreover, our findings suggest that lack of benefit seen in the St Francis Heart Study is not attributable to the modifying effects of antioxidant vitamins.

When compared with electron beam computed tomography, the accuracy of helical computed tomography in detecting coronary artery calcification has been questioned (Carr, Crouse et al. 2000) (Qanadli, Mesurolle et al. 2001). Technological advances have also meant that double-helical scanners have now been overtaken by 64 slice scanners. At trial inception, the double-helix scanner was "state-of-the-art" and it would have been inappropriate to replace the scanner during the conduct of the trial. Moreover, our approach has been previously validated and we have demonstrated good reproducibility of coronary artery calcification scores in patients with scores of greater

than 100 AU. We do not believe the absence of a major beneficial effect on coronary artery calcification is attributable to our methodology. We acknowledge the fact that our population size is modest; however, the 95% confidence intervals are able to exclude a relative reduction in progression of coronary artery calcification of >3%/yr. We therefore suggest that if lipid-lowering therapy does reduce the progression of coronary artery calcification then the effect is rather small.

Controversy exists over the method of quantification of coronary artery calcification. The Agatston method is traditionally employed but this may overestimate the coronary calcium score in newer generation scanners with reduced slice thickness due to partial voluming. More recent methods include the volume (Callister, Cooil et al. 1998) and the coronary calcium mass (Hong, Becker et al. 2002) scores, although neither are superior to the Agatston score in terms of reproducibility from consecutive scans in an individual patient (Rumberger and Kaufman 2003).

4.5.2 Conclusion

We conclude that intensive lipid lowering therapy does not halt the progression, or induce regression, of coronary artery calcification. Although coronary artery calcium scores correlate well with the presence of atherosclerosis and predict future coronary risk, our findings confirm that there is currently no role for monitoring progression of coronary artery calcification in order to assess the response to lipid lowering therapy.

CHAPTER 5

NON-INVASIVE ASSESSMENT OF CORONARY ARTERY BYPASS GRAFT PATENCY USING COMPUTED TOMOGRAPHY ANGIOGRAPHY

Houslay ES, Lawton T, Sengupta A, Uren NG, McKillop G, Newby DE. Non-invasive assessment of coronary artery bypass graft patency using 16-slice computed tomography angiography. *J Cardiothorac Surg.* June 5;**2**(1):27.

5.1 ABSTRACT

Background Invasive coronary angiography is the gold standard means of imaging bypass vessels and carries a small but potentially serious risk of local vascular complications, including myocardial infarction, stroke and death.

Aim To evaluate computed tomography as a non-invasive means of assessing graft patency.

Design Comparative observational study.

Methods Fifty patients with previous coronary artery bypass surgery who were listed for diagnostic coronary angiography underwent contrast enhanced computed tomography angiography using a 16-slice multidetector computed tomography scanner. Images were retrospectively gated to the electrocardiogram and two dimensional axial, multiplanar and three dimensional reconstructions acquired. Sensitivity, specificity, positive and negative predictive value, accuracy and level of agreement for detection of graft patency by multidetector computed tomography.

Results A total of 116 grafts were suitable for analysis. The specificity of CT for the detection of graft patency was 100%, with a sensitivity of 92.8%, positive predictive value 100%, negative predictive value 85.8% and an accuracy of 94.8%. The kappa value of agreement between the two means of measuring graft patency was 0.9. Mean radiation dose for coronary angiography was 9.0±7.2 mSv and for computed tomography was 18.5±4 mSv. Pooled analysis of eight studies, incorporating 932 grafts, confirmed a 96% accuracy for the detection of graft patency by multidetector computed tomography.

Conclusions Computed tomography is an accurate, rapid and non-invasive method of assessing coronary artery bypass graft patency. However, this was achieved at the expense of an increase in radiation dose.

5.2 INTRODUCTION

Coronary artery bypass grafting (CABG) was first performed in 1967 by Garrett et al,(Garrett, Dennis et al. 1973) who successfully employed a saphenous vein graft (SVG) for the treatment of coronary artery disease. This procedure has now become a widespread treatment for intractable angina and, in high risk patients, improves survival.(Davis, Chaitman et al. 1995) However, the benefits of surgery may be lost with graft failure or occlusion. Vein graft patency has been found to be reduced to 81% at one year, 75% at 5 years and less than 50% at 15 years.(Fitzgibbon, Kafka et al. 1996) This has led to the increasing use of arterial conduits, such as left internal mammary grafts (LIMA), that are associated with improved long term (10-15 year) patency and survival.(Barner, Standeven et al. 1985; Cameron, Davis et al. 1996)

Vein graft occlusion may occur early or late and is due to three distinct, well described disease processes. Acute graft failure and thrombosis may occur in the first 30 days postoperatively, and affects up to 12% of vein grafts.(Fitzgibbon, Kafka et al. 1996) Neointimal hyperplasia occurs between one month and a year, and is the result of accumulation of smooth muscle cells and extracellular matrix in the intimal compartment. While this rarely causes clinically significant stenosis,(Lie, Lawrie et al. 1977) it provides the foundation for the development of graft atheroma. Late graft failure results from an accelerated form of atherosclerosis called 'graft vasculopathy'. This process predominates beyond the first year after surgery and is present in 17% of grafts at 6 years and 46% of grafts at 11 years.(Bourassa, Fisher et al. 1985)

The gold standard method of assessing graft patency is coronary angiography. This invasive procedure carries the small risk (10 in 1000) of potentially serious local vascular complications, including myocardial infarction, stroke and death. Studies have shown that elective coronary angiography in clinically stable patients with saphenous vein grafts carries a 0.08% risk of myocardial infarction, while 0.7% of subjects experienced clinically important complications. The risk of myocardial infarction increased to 1.3% for urgent studies.(Gobel, Stewart et al. 1998) The assessment of graft patency in a non-invasive readily applicable manner would have major benefits for the management and treatment of patients with prior CABG. The large calibre and more static location of bypass grafts make them particularly suitable for investigation by potential non-invasive imaging modalities. Computed tomography angiography was first described as a means of determining bypass graft patency in 1980.(Brundage, Lipton et al. 1980; Guthaner, Brody et al. 1980) With advances in spiral and multidetector computed tomography (MDCT) technology, there has emerged a growing body of evidence to support the use of computed tomography for non-invasive bypass graft assessment.(Engelmann, von Smekal et al. 1997; Engelmann, Knez et al. 2000; Nieman, Oudkerk et al. 2001; Burgstahler, Kuettner et al. 2003; Dewey, Lembcke et al. 2004; Marano, Storto et al. 2004; Schlosser, Konorza et al. 2004; Willmann, Weishaupt et al. 2004; Song, Ito et al. 2005) Using the reference gold-standard of invasive coronary angiography, we aimed to assess whether contrast enhanced MDCT can reliably predict graft patency in patients who have previously undergone CABG.

5.3 METHODS

5.3.1 Patient population

Fifty consecutive patients who had undergone previous coronary artery bypass graft surgery and were listed for diagnostic coronary angiography between June 2004 and June 2005 were recruited into the study. Exclusion criteria were the presence of implanted metallic cardiac devices which may interfere with image quality (prosthetic heart valves, implantable pacemaker or cardiodefibrillator), renal impairment, atrial fibrillation or those patients unable to tolerate the supine position. The study was conducted with the approval of the local research ethics committee, in accordance with the Declaration of Helsinki and the written informed consent of each subject.

5.3.2 Coronary Angiogram

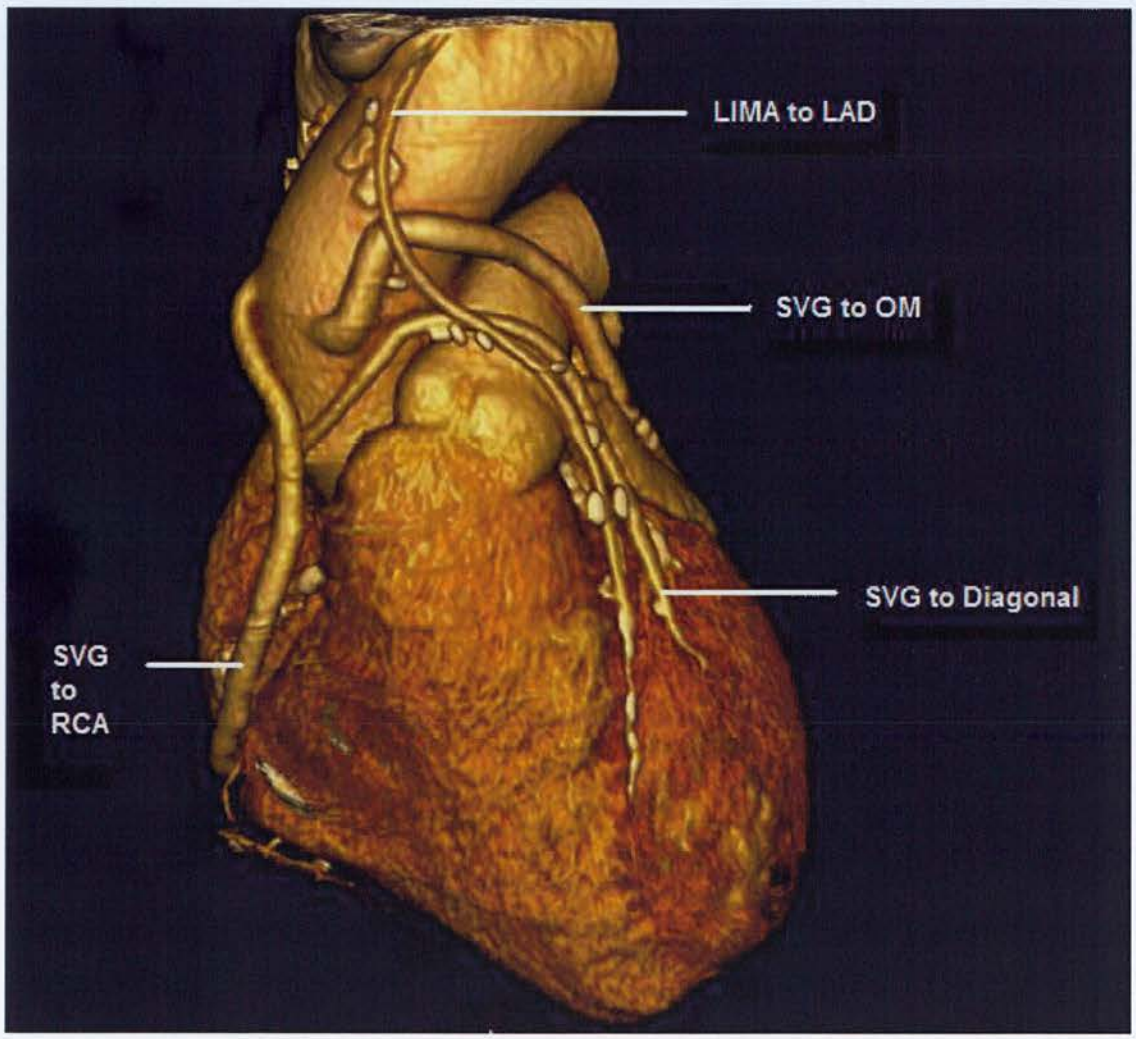
Coronary angiography was performed by an experienced cardiologist via standard percutaneous approach, using 6 French Judkins catheters. Images were obtained on a Innova digital flat plate system (Advantx, GE Medical Systems) following i.v. bolus injection of Niopam contrast agent (Bracco, Bucks, UK). Selective catheterisation of grafts or graft stumps was performed.

5.3.3 MDCT Angiogram

MDCT angiography was performed using a 16-slice MDCT scanner (Aquilon; Toshiba, Tustin, CA). The scan volume was defined based on expected location of the coronary arteries and grafts, following a scout view. In patients with known internal mammary

artery grafts, this area was extended to the origin of the IMA at the proximal subclavian arteries. Fixed scanning variables included a gantry rotation time of 0.5 seconds, 16 x 1 mm detector collimation, 0.35 to 0.5 x 0.35 to 0.5 mm pixel size, 135kV, 250 to 300 mA, 0.25 pitch, and inspiratory breath hold time 20-30 seconds. Iomeprol, 100 mL (400-strength, Bracco, Bucks, UK) contrast agent was administered intravenously. The ^{SURE}Cardio acquisition feature was utilised, which monitors the patient's heart rate for five consecutive beats, calculates an average and automatically selects optimal scan parameters, in order to optimise intravenous contrast concentration at the time of image acquisition. The images were obtained during inspiratory breath hold and retrospectively cardiac gated. The reconstruction set was limited to one phase at 75% of the R-R interval. From these images, one slice was selected to demonstrate best the three main coronary arteries at the mid-heart level. The selected slice was then reconstructed for the entire cardiac cycle at 20 ms intervals. From these images, the phase which best demonstrated the coronary arteries at this slice position were selected and the entire volume reconstructed at the selected phase (Figure 5.1). The images were transferred to a dedicated workstation (Vitrea v3.5; Vital Images, Plymouth, MN).

Figure 5.1 Three dimensional reconstruction with volume rendering techniques demonstrating saphenous vein grafts (SVG) to the posterior descending branch of the right coronary artery (RCA) and obtuse marginal branch of the circumflex artery (OM) and left internal mammary graft (LIMA) to left anterior descending artery (LAD).



5.3.4 Data Analysis

Two-dimensional axial, multiplanar reconstruction and three-dimensional reconstructions with volume rendering techniques were analysed by a radiologist who was familiar with the cardiac anatomy but blinded to the result of the coronary angiogram. Image quality was graded in terms of eligible or insufficient (motion artefact, artefact caused by surgical clip) and eligible grafts were assessed in terms of patency and the presence and location of significant stenoses (stenosis >50% luminal diameter) were noted. The results of MDCT were compared with coronary angiography, the gold standard reference. Sensitivity, specificity, positive and negative predictive value (PPV and NPV respectively) was calculated for the detection of graft patency by MDCT. Radiation dose for invasive coronary angiography and MDCT angiography was calculated from the documented dose area product ($cGycm^2$) and dose length product (mGycm) respectively.

5.4 RESULTS

Patients were predominantly male, with a mean graft age of 7±5 years (Table 5.1) and the majority (66%) were on beta-blockers. Mean heart rate was 67 beats per minute (range 52-89). MDCT angiography was performed on all 50 patients a mean of 55 days following conventional angiography (range 40-74). Of these patients, two studies were not of sufficient diagnostic quality due to movement artefact and technical failure, thereby excluding 6 grafts from analysis. A further six grafts were non-diagnostic on MDCT angiography and a further one excluded due to stent insertion with subsequent in-stent stenosis between recruitment to the study and time of MDCT. Of 129 grafts, a total of 116 were suitable for analysis. There were no complications as a result of the MDCT or invasive diagnostic angiography.

MDCT correctly identified 77 of 83 patent grafts and 33 of 33 occluded grafts (Table 5.2). The sensitivity for detection of graft patency was 92.8%, specificity 100%, positive predictive value 100%, negative predictive value 84.6% and accuracy 94.8%. The main discrepancies lie in the reporting of IMA grafts, where there were a significant proportion of false occlusions (Table 5.3). There was a very good strength of agreement between the two imaging modalities (Cohen's κ=0.9).

Table 5.1 Baseline Subject Characteristics

No. of patients	**50**
Sex (% male)	**86**
Mean age (SD)	**66 (9) years**
Heart Rate (Mean, Median, Range)	**67, 68, 52-89**
No. of bypass grafts	**116**
No. of IMA grafts	**35**
• IMA → LAD	30
• IMA → OM	2
• IMA → Diagonal	2
• IMA → Circumflex	1
No. of SVGs	**77**
• SVG → OM	26
• SVG → RCA	25
• SVG → LAD	13
• SVG → Diagonal	7
• SVG → posterior descending	6
Radial artery grafts	**4**
• → OM	3
• → posterior descending	1
Mean graft age (SD)	**7 (5) years**

Table 5.2 Graft characteristics

	Angiography		MDCT			
	patent	occluded	True patent	False patent	True occluded	False occluded
Overall n=116	83	33	77	0	33	6
IMA n=35	30	5	25	0	5	5
SVG n=77	52	25	51	0	25	1
Other n=4	1	3	1	0	3	0

Table 5.3 Diagnostic accuracy of MDCT for detection of graft patency

	Sensitivity % *(95% C.I.)*	Specificity % *(95% C.I.)*	PPV % *(95% C.I.)*	NPV % *(95% C.I.)*	Accuracy % *(95% C.I.)*
Overall n=116	**92.8** *(84.9 – 97.3)*	**100** *(91.3 – 100)*	**100** *(96.2 – 100)*	**84.6** *(69.5 – 94.1)*	**94.8** *(89.1 – 98.1)*
IMA grafts n=35	82.8	100	100	50	85.3
SVG n=77	98.1	100	100	96.3	98.7
Other n=4	100	100	100	100	100

5.4.1 Radiation exposure

The mean radiation exposure for coronary angiography was 9.0±7.2 mSv with mean screening time of 11±7.3 minutes. The mean radiation exposure for MDCT angiography was 18.5±4 mSv with a mean scan time of 24 seconds (range 18-26).

5.5 DISCUSSION

MDCT provides a highly specific means of detecting coronary artery bypass graft patency in a clinical setting. It is safe and abolishes the need for time consuming invasive coronary angiography which is associated with the small but significant risk of major complications. In contrast to invasive coronary angiography, it can be performed on a scheduled out-patient basis rather than requiring admission to a day case unit.

Computed tomography was first investigated as a possible method of determining patency of coronary artery bypass grafts in the early 1980s. Gunthaner et al(Guthaner, Brody et al. 1980) (1980) found that they were able to determine patency in 77.5% of left anterior descending (LAD) and right coronary artery (RCA) grafts and 40% of obtuse marginal (OM) grafts. Brundage et al(Brundage, Lipton et al. 1980) published results that year showing 95% correlation between single slice CT and conventional angiographic detection of graft patency. With the advent of spiral and MDCT scanners, image quality has improved and, in line with this, interest in minimally invasive imaging techniques has grown. Since these first studies, further larger scale studies have been undertaken to assess the ability of CT to assess graft patency. Recently, Song and colleagues reported that MDCT imaging resulted in 99.4% specificity and 100% sensitivity, with 100% PPV and 80% NPV for detection of bypass graft patency.(Song, Ito et al. 2005) This study, however, looked at patients (n=50) immediately post-coronary artery bypass operation, who may be expected to have a high patency rate and no graft vasculopathy, thereby minimising any ambiguity caused by poor flow in chronically diseased grafts. Recent studies (n=25-65) looking specifically at graft

patency in study populations similar to the present, have reported sensitivities of 90-98% and specificities of 88-100% for detection of graft patency.(Engelmann, von Smekal et al. 1997; Hoshi, Yamauchi et al. 2001; Marano, Storto et al. 2004) Consistent with our findings, detection of patency in vein grafts is more reliable than for internal mammary grafts.(Ha, Cho et al. 1999; Engelmann, Knez et al. 2000)

We performed a pooled analysis of all available data from studies investigating CT as a non-invasive means of assessing coronary artery bypass grafts (Table 5.4).(Achenbach, Moshage et al. 1997; Engelmann, von Smekal et al. 1997; Engelmann, Knez et al. 2000; Ropers, Ulzheimer et al. 2001; Burgstahler, Kuettner et al. 2003; Marano, Storto et al. 2004; Martuscelli, Romagnoli et al. 2004; Schlosser, Konorza et al. 2004; Chiurlia, Menozzi et al. 2005; Moore, Sampson et al. 2005) The paper by Nieman *et al* was not included in the analysis due to incomplete data on detection of graft occlusion by MDCT.(Nieman, Pattynama et al. 2003) The pooled data gave an overall sensitivity of 96% and specificity of 99% (n=1498) for the detection of graft patency. As we have observed, studies with a preponderance of vein grafts displayed more reliable patency rates than those with a large proportion of arterial grafts. Pooled analysis focussing on the breakdown of available data for IMAs (n=268) gave a sensitivity of 93%, specificity 97%, PPV 99.5%, NPV 67% and an accuracy of 94%.(Engelmann, von Smekal et al. 1997; Engelmann, Knez et al. 2000; Ko, Choi et al. 2003; Marano, Storto et al. 2004; Schlosser, Konorza et al. 2004) Pooled analysis of vein grafts in the same studies (n=399) gave a sensitivity of 97%, specificity 97%, PPV 99%, NPV 92% and an accuracy of 97%.

Table 5.4 Characteristics of studies assessing non-invasive imaging of coronary artery bypass grafts.

	Achenbach *et al*	**Engleman** *et al*	**Engleman** *et al*	**Ropers** *et al*	**Ko** *et al*	**Marano** *et al*	**Schlosser** *et al*	**Martuscelli** *et al*	**Moore** *et al*	**Chiurlia** *et al*	**Houslay** *et al*	*Pooled data*
Year of publication	1997	1997	2000	2001	2003	2004	2004	2004	2005	2005	2005	
Method	Electron beam CT	Spiral CT	Spiral CT	Spiral CT	4 slice MDCT	4 slice MDCT	16 slice MDCT	16 slice MDCT	4 slice MDCT	16 slice MDCT	16 slice MDCT	
No. of patients	25	49	24	65	39	57	51	96	50	52	50	
Mean age	64	61	61	67	60	65	65	62	69	63	66	
Sex (% male)	92	92	92	82	74	95	76	83%	76	87	86	
No. grafts	**54**	**134**	**78**	**182**	**115**	**122**	**131**	**251**	**150**	**165**	**116**	*1498*
IMA	1	42	20	20	40	95	40	85	38	46	35	*462*
SVG	53	92	58	162	54	27	91	166	112	117	77	*1009*
other					21					2	4	27
% vein grafts	98%	69%	74%	89%	47%	22%	69%	66%	66%	70%	66%	
Mean graft age (yrs)	6.9	1.8	2.1	7.6	1.2	4.8	5.6	7	8	7.9	7.0	
Sensitivity	100%	92%	90.1%	97%	93%	93%	96%	100%	91%	100%	93%	***96%***
Specificity	100%	97%	100%	98%	99%	98%	95%	100%	100%	100%	100%	***99%***
PPV	100%	99%	100%	97%	93%	93%	81%	100%	100%	100%	100%	***99%***
NPV	100%	78%	74%	98%	99%	98%	99%	100%	96%	100%	85%	***95%***
Accuracy	100%	93%	92%	98%	98%	97%	95%	100%	97%	100%	95%	***97%***

Computed tomography has many advantages over coronary angiography, including a lower complication rate, better ostial imaging and easy visualisation of vessels with anomalous origin and those where catheterisation has failed. MDCT in particular has the advantage of shorter breath hold times, faster gantry rotation and reduced slice thickness permitting better temporal and spatial resolution than previous CT scanners. MDCT is widely available in standard Radiology departments, unlike electron beam CT.

The main limitation of CT imaging is the high radiation doses that are incurred. This may be of limited relevance in the population under question as it has been shown that the elderly are less susceptible to the lifetime risk of radiation exposure for any given dose.(Pierce, Shimizu et al. 1996) Nonetheless, the radiation dose is double that of conventional angiography. Whilst there are no national dose limits, the doses such as are incurred with CT angiography equate to more than eight times the annual natural background radiation exposure.

Whilst CT angiography has shown promising results in the detection of bypass graft patency, a recent review article concluded that there is a lack of evidence-based data to support its use for evaluation of native vessels in patients presenting with chest pain (Schoenhagen 2007). CT angiography may be a helpful non-invasive imaging tool in evaluation of bypass grafts on a non-urgent outpatient basis, but clinical trials are needed before it can be used as a tool to evaluate native vessels in patients presenting acutely.

5.5.1 Study Limitations

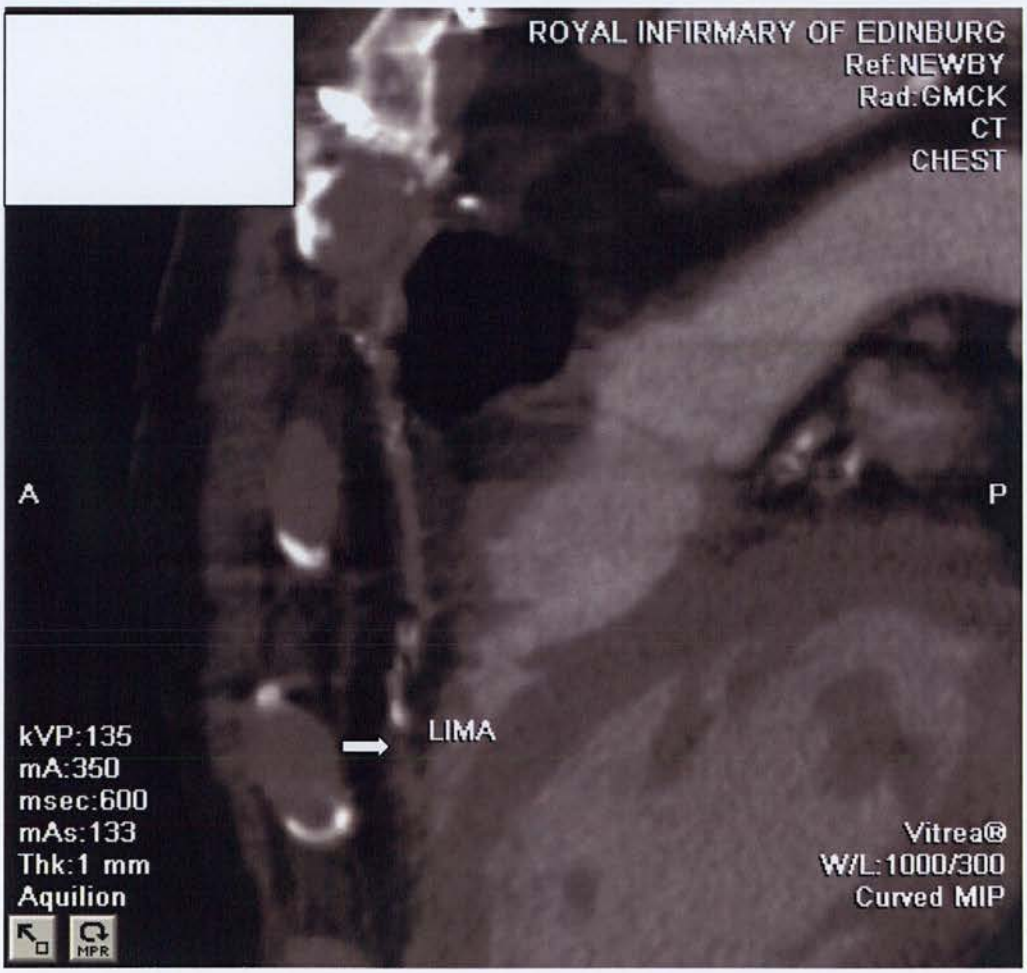
Image quality is highly dependent upon adequate heart rate control, with a great reduction in image quality in subjects with a heart rate of greater than 70 beats per minute, due to diastolic motion artefacts. While bypass grafts are less susceptible to cardiac motion artefacts than native vessels, this can still be an issue (Fig. 5.2), in particular in posterior vessels, such as grafts to the circumflex artery. Not all of the patients who were recruited were on β-blockade therapy, which meant that image quality was in some cases suboptimal due to diastolic motion artefacts. In those patients who had IMA grafts, the increased scan volume necessitated a long breath hold. In some patients, this led to a degree of respiratory artefacts towards the end of the scan time, which tended to coincide with the base of the heart and therefore the distal graft anastamoses. For this reason, we were also unable to assess the distal vessel 'run off' consistently. In future studies, this could be addressed by administering short acting β-blockers immediately prior to the scan.

In clinical practice, it is helpful to have information as to the severity of stenosis in a vessel so as to plan revascularistion therapy. Due to motion artefact and artefact from surgical clips in some patients, we were unable to reliably assess stenosis severity. In addition, the raw data from the scans was lost, so we were unable to reconstruct the images at different points in the cardiac cycles, which may have been helpful in the assessment of each individual vessel.

The use of 16 slice CT scanners has been superseded by newer 64 slice scanners, which have the advantage of narrow collimation and reduced scan times. Meyer et al

report reliable assessment of graft patency and stenoses in unselected populations using 64 slice scanners (Meyer, Martinoff et al. 2007). Furthermore, Zhang et al highlight improved rated of evaluation of proximal anastamosis, graft, distal anastamosis and run off vessel for 64 slice scanners in comparison to 16 slice CT (Zhang, Jin et al. 2006).

Figure 5.2 Multiplanar reformat



Multiplanar reformatted image showing considerable motion artefact. The LIMA was judged to be occluded distally on MDCT (arrow). This was, however, patent on conventional coronary angiogram and is an example of how motion artefact can lead to a falsely occluded diagnosis at MDCT.

5.5.2 Conclusions

In line with recent studies, we can confirm that MDCT is highly specific for the detection of bypass graft patency. It provides a safe, fast and cost effective means of imaging coronary artery bypass grafts but with the disadvantage of a high radiation dose. The introduction of new 64 slice MDCT scanners will result in further improved image quality which may enable non-invasive imaging to be the mainstay of assessment of graft patency in the future.

CHAPTER 6

NOVEL MAGNETIC RESONANCE IMAGING TECHNIQUES AND REMODELLING OF COMPLEX CAROTID ATHEROSCLEROSIS:

A FEASIBILITY AND REPRODUCIBILITY PILOT STUDY

6.1 BACKGROUND

Cardiovascular disease is the commonest cause of premature death in Scotland and leads to substantial morbidity. Recent estimates put the prevalence of stroke in Scotland at 2.33 per 1000 (Alexander, Bugge et al. 2000).

The principal initiating event in the pathogenesis of acute cardiovascular events is rupture or erosion of the atherosclerotic plaque (Davies 2000). This can lead to intravascular thrombus formation and the potential for acute vessel occlusion or thromboembolism. The resolution or progression of unstable plaques determines the immediate consequence of plaque rupture and the resultant clinical event experienced by the patient: a fifth of patients presenting with a transient ischaemic attack or minor stroke will have a subsequent major stroke (Flossmann and Rothwell 2003). Insights into the natural history of these acute plaque events are therefore fundamental to the understanding and treatment of acute cardiovascular disease.

The examination of the carotid arteries of patients with recent cerebral ischaemia, provides an ideal opportunity to identify key features in acute plaque events. High resolution imaging of coronary arteries is currently only possible with invasive techniques, such as intravascular ultrasound (Newby, McLeod et al. 2001; McLeod, Newby et al. 2003). Moreover, ultrasound imaging of complex atherosclerotic plaques is limited by the poor resolution of in situ thrombus, potential distortion of geometry and the inability to image through calcified plaques. Non-invasive techniques, such as magnetic resonance imaging, hold promise, but applicability to the coronary circulation is limited by the relatively small calibre of the coronary vasculature and the movement artefact induced by continuous myocardial contraction.

Carotid arteries are more suitable for non-invasive imaging because of their greater calibre, superficial location and relatively static position. This vessel therefore lends itself more readily to the assessment of acute plaque events and subsequent remodelling.

Magnetic resonance provides a means of non-invasive imaging without the need for ionising radiation and, with the use of dedicated surface coils, can produce high-resolution images of carotid atherosclerotic plaques. Recent novel and exciting MR techniques can facilitate characterisation of complex atherosclerotic plaques. T_1-, proton density and T_2-weighted images and three-dimensional time-of-flight images can be used to identify complex plaque with surface defects, haemorrhage or thrombus (Cai, Hatsukami et al. 2002; Yuan, Zhang et al. 2002). The neovasculature and vasa vasorum are part of the inflammatory process in complex atherosclerotic plaques. Using the standard MR contrast agent gadolinium, it has been possible to image and quantify neovascularisation of carotid plaques in patients against subsequent histomorphometric analysis of carotid endarterectomy specimens (Kerwin, Hooker et al. 2003).

The description of *in vivo* atherosclerotic plaque remodelling may have widespread implications for understanding the mechanisms and treatment of atherosclerotic disease including not only cerebrovascular disease but also coronary artery disease and peripheral vascular disease.

6.2 METHODOLOGY

6.2.1 Patients

Fifteen patients with recent symptoms and signs of an acute transient ischaemic attack, amaurosis fugax or stroke were recruited from the acute neurovascular clinic at the Western General Hospital and the vascular surgical clinic at the Royal Infirmary of Edinburgh. Informed consent was obtained from all patients, and the study was approved by the regional ethics committee. All patients underwent careful clinical evaluation. Subsequent retention in the study was dictated by a Doppler ultrasound of the ipsilateral internal carotid artery which confirmed a >40% luminal stenosis (on ECST criteria), to account for their clinical symptoms and presentation. Exclusion criteria were women of child bearing potential, renal or hepatic failure, severe or significant co-morbidity, or if unable to tolerate the supine position.

6.2.2 Magnetic Resonance Imaging

In order to assess feasibility, each subject underwent the following magnetic resonance imaging protocol. Subjects were placed in a head collar to reduce movement artefact. Carotid surface coils (bilateral four channel phased array) were applied to maximise resolution, and scanning was cardiac synchronised to minimise pulsation artefacts. A research dedicated magnetic resonance imaging scanner (1.5T GE Echospeed) performed a two dimensional time of flight (2D TOF) localiser view with a field of view (FOV) of 22 cm. The carotid bifurcation was identified from the localiser sequence and subsequent sequences, based around the carotid bifurcation, included axial T1 DIR 'black blood' with a FOV of 14 cm; proton density and T2 fast spin echo (FSE) with FOV of 13 cm; and 3D TOF with FOV of 13 cm before and after enhancement with an intravenous bolus of 0.1 mmol/kg Dotarem® (Gadoterate

meglumine, Guerbet S.A., Paris, France). For each sequence, this resulted in 9 slices, each 2mm thick, centred upon the carotid bifurcation of interest, thus giving coverage of 1.8 cm. In order to assess reproducibility, the imaging protocol was then repeated within 2 weeks of the first assessment. Measurements of plaque area and volume were then compared between the two sets of scans.

In order to assess neovascularisation, four subjects underwent an initial DIR axial scan (11 slices, 2mm thick), followed by a fast spoiled gradient recalled echo sequence (FSPGR), with T-1 weighted images of the diseased carotid artery at 9 locations, centred on the carotid bifurcation. Following the method of Kerwin *et al*, 2003, each acquisition was repeated 10 times, with a repetition interval of 16 seconds. Coincident with the second image in the sequence, 0.1 mmol/kg of Dotarem® was injected at a rate of 2mL/s via a power injector.

6.2.3 Quantification of Carotid MR images

Through collaboration with the Image Analysis Core of the Wellcome Trust Clinical Research Facility, digital images were quantitatively assessed. Using existing software, the cross-sectional lumen and outer vessel wall boundaries were manually traced and cross-sectional areas calculated [Fig 6.1]. Plaque area was defined as the vessel area minus the lumen area. Overall plaque area and volume were measured with reference to the carotid bifurcation to ensure consistency and comparability of study segments. Variation in pixel intensities within the plaque was also assessed. All scans, prior to measurements, were set at the same intensity level (Level 1111, Width 222). A software package was then used to calculate the occurrences of different intensity levels within the plaque. Histograms of occurrences against

intensity were then generated, with the hypothesis that heterogeneous plaques would yield differing histograms to homogeneous plaques.

Finally, neovascularisation was determined by the degree of gadolinium enhancement of the plaque. After the acquisition of the 9 locations around the carotid bifurcation, the area of the plaque on each location was manually traced as described above. These regions of interest (plaque areas) were then aligned for each of the 11 time points and analysed for changes in signal intensity over time.

6.2.4 Power Calculation and Statistical Analysis

This was a preliminary study which aimed to establish the feasibility and reproducibility of novel magnetic resonance based techniques. An accurate estimate of statistical power was not possible. As a pilot study, the data generated will permit a formal power calculation to be made for potential future clinical trials. For the parametric and volumetric variables described above, reproducibility was assessed by the method of Bland and Altman,(Bland and Altman 1986) and a paired students t-test performed. The correlation between MRI and histological plaque grading, was assessed using the Kappa statistic.

6.3 RESULTS

6.3.1 Feasibility and patient demographics

A total of 15 patients were recruited. Eleven underwent the standard protocol described above. A further 4 patients (subjects 12-15) were recruited to undergo the neovascularisation protocol. The percentage of internal carotid artery stenosis on ultrasound, ranged from 48-90%. The time from symptoms to recruitment and initial scan was between 6 and 60 days. The time between first and second scans ranged from 1 to 7 days (table 6.1).

Table 6.1 Patient parameters

Patient	Affected side	Duplex % Stenosis	Symptoms	Symptom to scan time (days)	Time between scans (days)
1	L	56	R arm weakness	6	1
2	R	69	L arm weakness	14	7
3	R	80	L arm weakness	30	7
4	L	53	Expressive dysphasia	10	1
5	L	90	Beurgers disease; RICA occluded		1
6	R	48	R amaurosis fugax	23	1
7	R	70	R amaurosis fugax	60	2
8	L	70	R arm weakness	20	2
9	R	70	R amaurosis fugax	60	1
10	R	85	L arm weakness	41	2
11	R	70	L arm weakness	53	1
12	L	80	R arm weakness, dysarthria	48	1
13	L	80	Expressive dysphasia	46	1
14	R	80	L arm weakness	14	1
15	L	80	L amaurosis fugax	28	1

6.3.2 Reproducibility

Measurements of plaque area and volume were conducted on the two T1 weighted images before and after contrast agent Gadolinium. Although images were also obtained using T2 weighted, Proton Density and 3-D TOF scanning sequences, these were judged by 2 independent radiologists to be of unsatisfactory image quality for

the performance of structural measurements. It was thought that the longer duration of these sequences made them more sensitive to movement artefact. (Fig 6.2)

Plaque area and volume measurements were generated for each slice. However, the mean measurement for all 9 slices was used for Bland Altman comparison as this was thought to be the most clinically relevant summary measurement. There were no significant differences between T1 weighted measurements of plaque area or volume made on scan one or two (Figs. 6.3). Although there was no statistically significant difference in measurements when contrast agent was used, the confidence intervals were noted to be wider (Figs. 6.4)

6.3.3 Plaque Characteristics

Previous studies have used MRI to identify various components of carotid plaque by comparing MRI images around the carotid bifurcation, with matched histological sections. Cai *et al* produced a table of modified MRI classification for different types of atherosclerotic plaque (Table 2.3). These gradings were applied to each scan (Table 6.3).

The occurrence of different intensity signals within the plaque was plotted as a histogram. Lipid gives a high signal on a T1-weighted scan, whereas fibrous tissue produces an intermediate signal. It was hypothesised that a complex plaque consisting of several components (lipid, haemorrhage, fibrous tissue), would produce a range of signal intensities, and hence a wider or bimodal histogram (Fig. 6.5). Fig. 6.6 illustrates the relationship between the standard deviation of the intensity histogram for the internal carotid slice and overall MRI grading of the scan.

Table 6.3

Modified AHA Classification for MRI	No. of patients
I	0
II	0
III	3
IV	0
IV-V	3
V	0
VI	4
VII	1

6.3.3 Neovascularisation

Four patients underwent a neovascularisation sequence to assess the feasibility of previously reported methodology [Kerwin *et al*]. Fig. 6.7 demonstrates the change in intensity over time following intravenous injection of contrast agent gadolinium. The change in intensity signal in the plaque follows that of the jugular vein. Fig. 6.8 depicts images of the common carotid artery and jugular vein at the same location, over progressive time points. Image 1 is just before contrast injection, and the jugular vein appears dark. Image 2 is following intravenous contrast and the vein appears bright. Over time, the dark rim of plaque in the carotid artery progressively brightens. In future, the aim is to confirm that the areas of changing signal intensity seen within the plaque on MRI correspond with regions of neovascularisation by performing a comparative study with histological specimens in patients undergoing carotid endarterectomy.

6.4 DISCUSSION

Previous reports have claimed that it is feasible to image atherosclerotic plaque in carotid arteries using MRI (Yuan, Mitsumori et al. 2001). This project demonstrated that our centre was capable of employing these protocols in a similar fashion. Although the aim was to scan patients within 7-14 days of initial symptoms, the results demonstrate a time from symptoms to recruitment that varies between 6 and 60 days. Patients were recruited from the acute neurovascular clinic; Lothian NHS guidelines state that patients referred for outpatient follow up and investigation following suspected TIA, should be seen within 2 weeks. However, despite this guideline, experience was that patients attended the clinic anywhere from 1-12 weeks after their initial presentation. The delay from symptoms to scan, was due to late referral to clinic. This time frame does however correlate with other studies which report scans conducted within 70-90 day of symptoms.

Previous reports have used an MRI protocol of at least 4 contrast weightings (T1-, PD-, and T2-weighted and 3D TOF) to image carotid plaque (Yuan, Mitsumori et al. 2001). Although it has been suggested that the use of different contrast weightings can help classify different plaque components, the overall image quality varied greatly between different contrast weightings (Fig. 6.2). In this study, the T1-DIR sequence yielded the best quality images which correlates with other studies that demonstrate lower variability between measurements performed on T1 weighted images compared with other sequences (Yuan, Beach et al. 1998). Although more information may be gleaned from a multi-weighted protocol, it was found that the inevitable increase in scanning time led to increased patient restlessness and a subsequent reduction in image quality due to movement artefact. In conclusion, although it may be feasible to

perform a 4 weightings sequence, it may result in the sacrifice of image accuracy, which is important when addressing small areas of interest.

There was good reproducibility between scans performed at 2 separate time points. The mean difference in plaque area (scan 2 minus scan 1) was -0.56± 5.0 mm^2 for T1-weighted scans *pre*-gadolinium (P=0.22) and -3.57±15.3 mm^2 for T1-weighted scans *post*-gadolinium (P=0.59) (Fig. 6.3). These results compare favourably with previously published reproducibility figures (Yuan, Beach et al. 1998). Although there was no statistically significant difference in measurements performed on scans post contrast injection (plaque area post gadolinium P=0.5, plaque volume post gadolinium P=0.5), the confidence intervals were noticeable wider (plaque area *pre*-adolinium 95% CI -3.9 to 2.8; Plaque area *post*-gadolinium 95% CI -15 to 8.2). It is interesting that the use of contrast agent should lead to a greater variation in scan measurements. Bland Altman comparison of plaque measurements, made with and without contrast agent, demonstrates a tendency for the use of contrast to lead to overestimation in plaque size (Fig. 6.9).

Another aim of this project was to familiarise our centre with the techniques of assessing plaque components. Certain features of plaque morphology (such as large lipid load and thin fibrous cap) are thought to increase the risk of plaque rupture and subsequent clinical sequelae. Previous studies have demonstrated the ability of MRI scanning to detect various morphological features of atherosclerotic plaques by the comparison of MRI images of carotid arteries with histological sections of the same artery following carotid endarterectomy. In this pilot study, a neuroradiologist graded the MRI scans using a previously reported criteria (Cai, Hatsukami et al. 2002). In

addition, a histogram of signal intensities within each plaque was plotted. Although the numbers were too small to achieve statistically significant differences, the trend is for a wider histogram with more complex plaques, i.e. a higher modified AHA classification. We hope to expand our study to include comparison with histological specimens in the future.

The early results from neovascularisation sequences are encouraging. The initial graphs of changing signal intensity over time correspond with those produced by Kerwin *et al* (Kerwin, Hooker et al. 2003). One of the subjects failed to demonstrate increasing signal intensity after intravenous contrast followed by signal decay as the contrast cleared. This patient had not tolerated the procedure well and found it difficult to remain still, leading to poorer quality images, difficulty in identifying regions of interest on the scan, and subsequent discrepancies in matching areas imaged at different time points. We aim to develop a smoothing protocol for our images with which to eliminate movement artefact, as used by Kerwin *et al.* This should help overcome some of these problems.

The main focus of this small pilot project was to assess the feasibility of conducting MR imaging of complex carotid plaques. It has allowed our centre to gain experience in this technique, and it has been demonstrated that this tool can be utilised in a reproducible fashion. This study provides essential pilot data that will allow aid in the planning of future projects.

6.4.1 Importance to NHS and possible implementation

The long-term consequences of cerebrovascular disease require the provision of substantial health care resources. The identification of high-risk patients and the potential to develop treatment strategies to prevent progression to cerebral infarction are essential goals in the treatment of patients with symptomatic carotid artery disease.

Clinically applicable non-invasive imaging modalities may provide a novel method of identifying those patients at greatest risk of developing acute stroke. Moreover, understanding the mechanisms of carotid plaque remodelling has the potential to develop novel therapeutic strategies to prevent progression to cerebral infarction and permanent disability. Finally, describing *in vivo* atherosclerotic plaque remodelling may have more widespread implications for understanding the mechanisms of generalised atherosclerotic disease including coronary artery disease and peripheral vascular disease. Magnetic resonance imaging may be capable of fulfilling these important roles.

6.4.2 Conclusions

We have shown that it is feasible to recruit patients with recent acute carotid plaque events for MRI scanning within an acceptable time frame. MR imaging of the carotid bifurcation using T1 weighted sequences has been used to provide statistically significant, reproducible information, similar to that reported in the literature. The tendency of contrast agent to increase the variability of measurements has been suggested, although this was not statistically significant. MRI features have been used to grade plaque morphology allowing correlation of plaque heterogeneity with

signal intensity heterogeneity as demonstrated by plotting histograms. Finally, we have been able to conduct a protocol for the demonstration of plaque neovascularisation, reproducing previously reported results.

Fig. 6.1 Lumen and vessel wall tracing

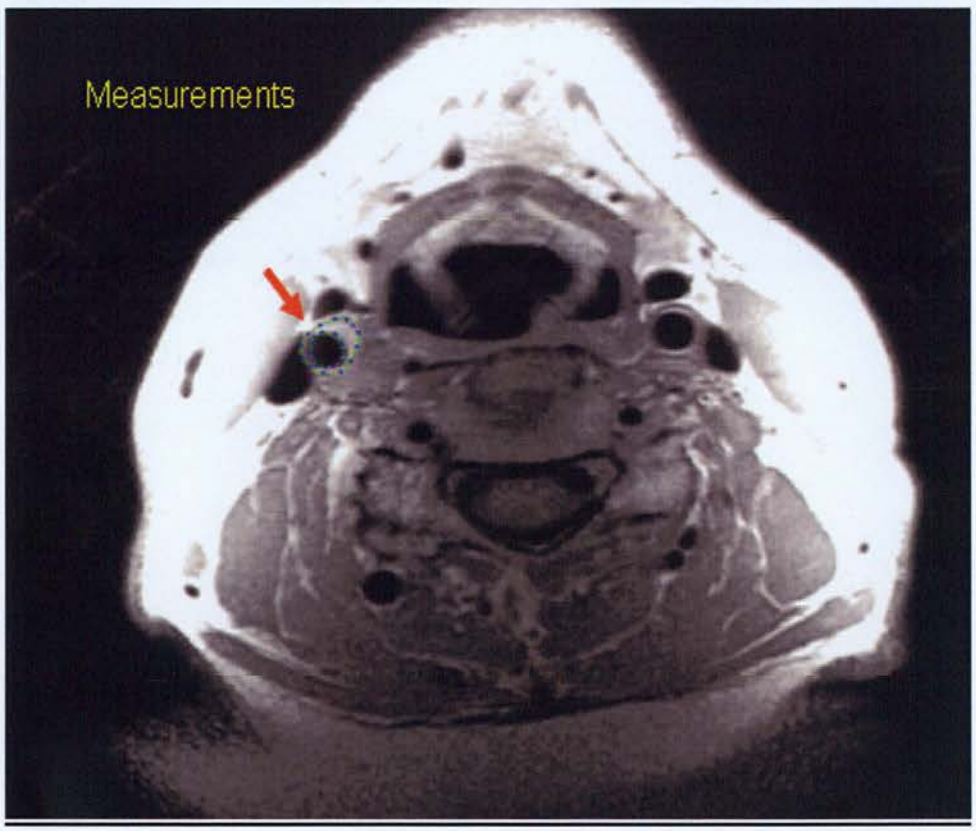
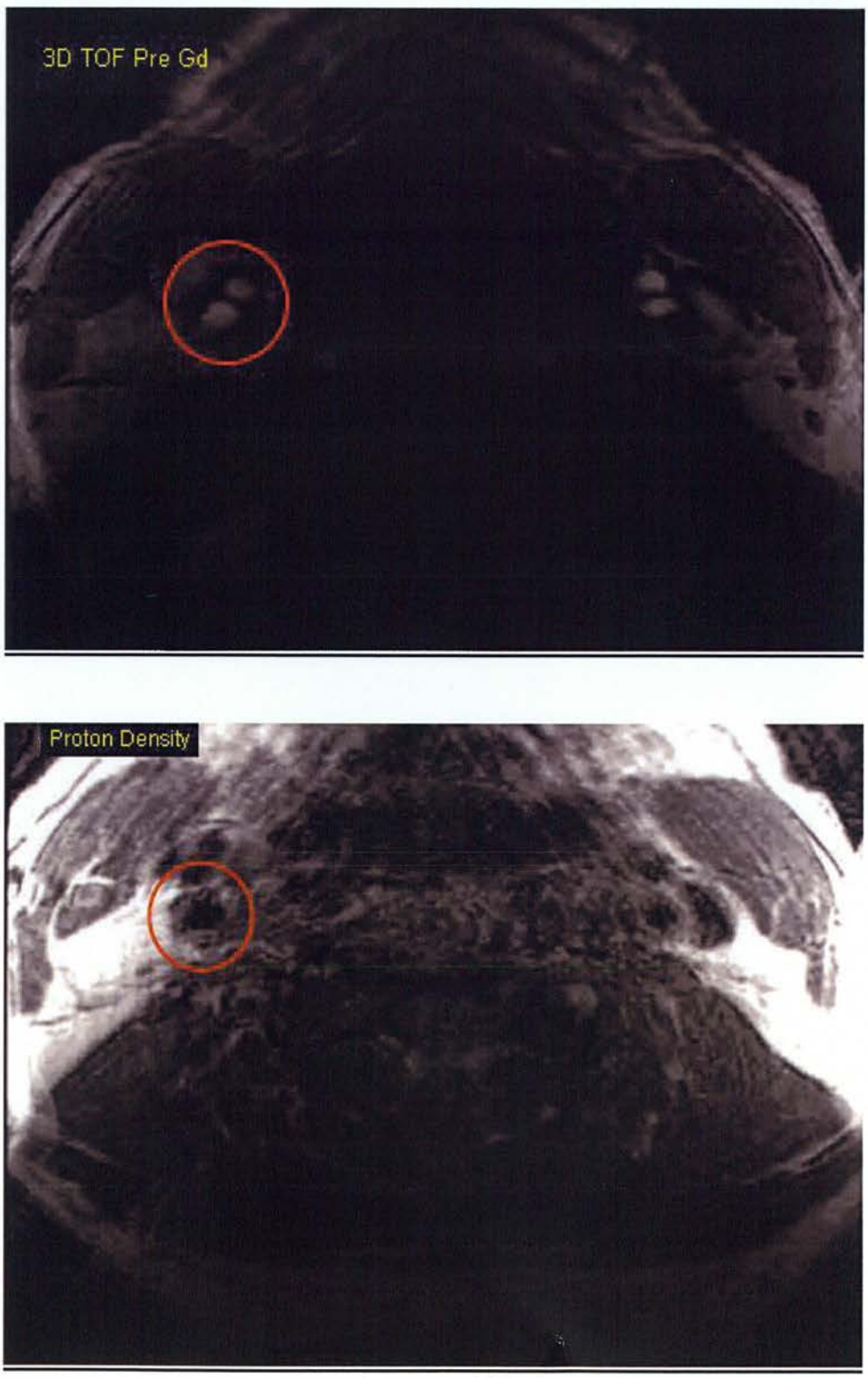
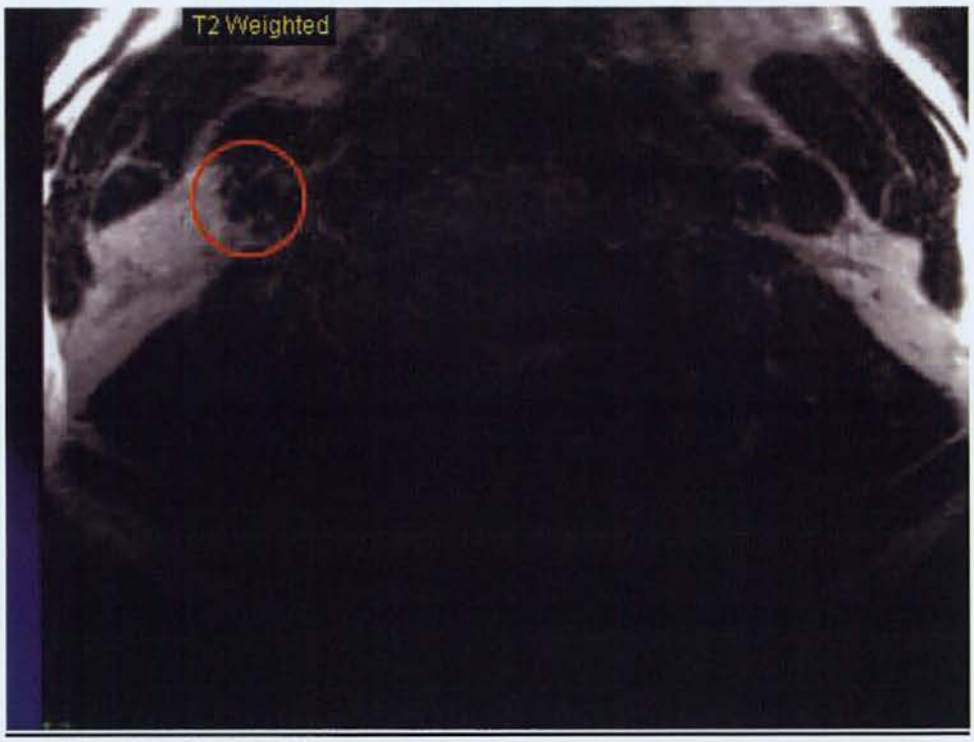


Fig. 6.2 MR sequences




T2 Weighted

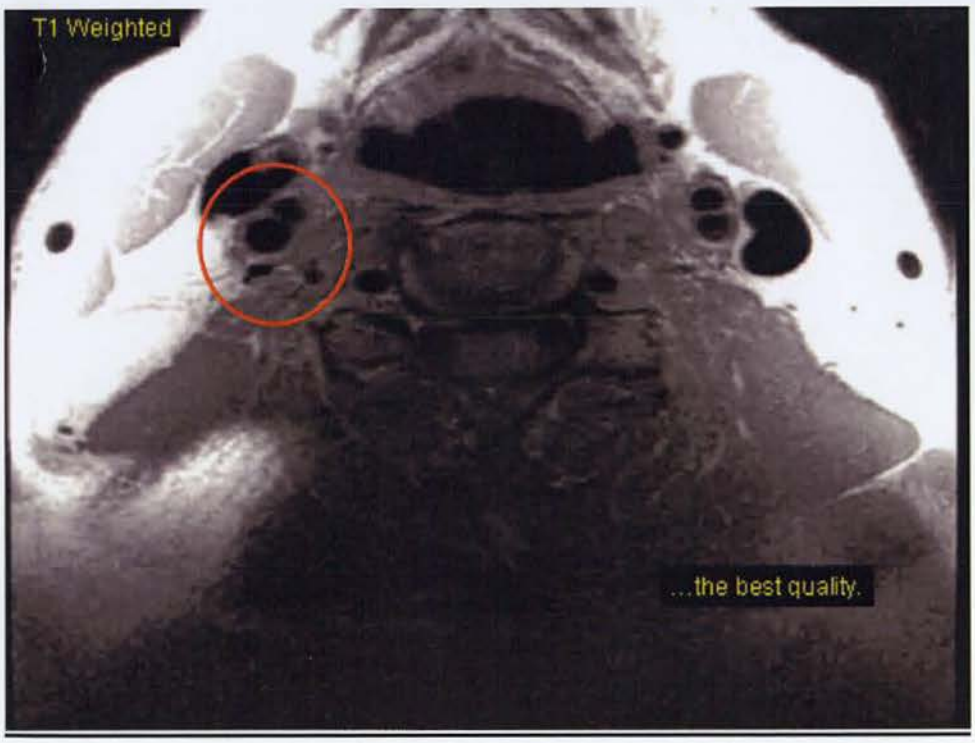
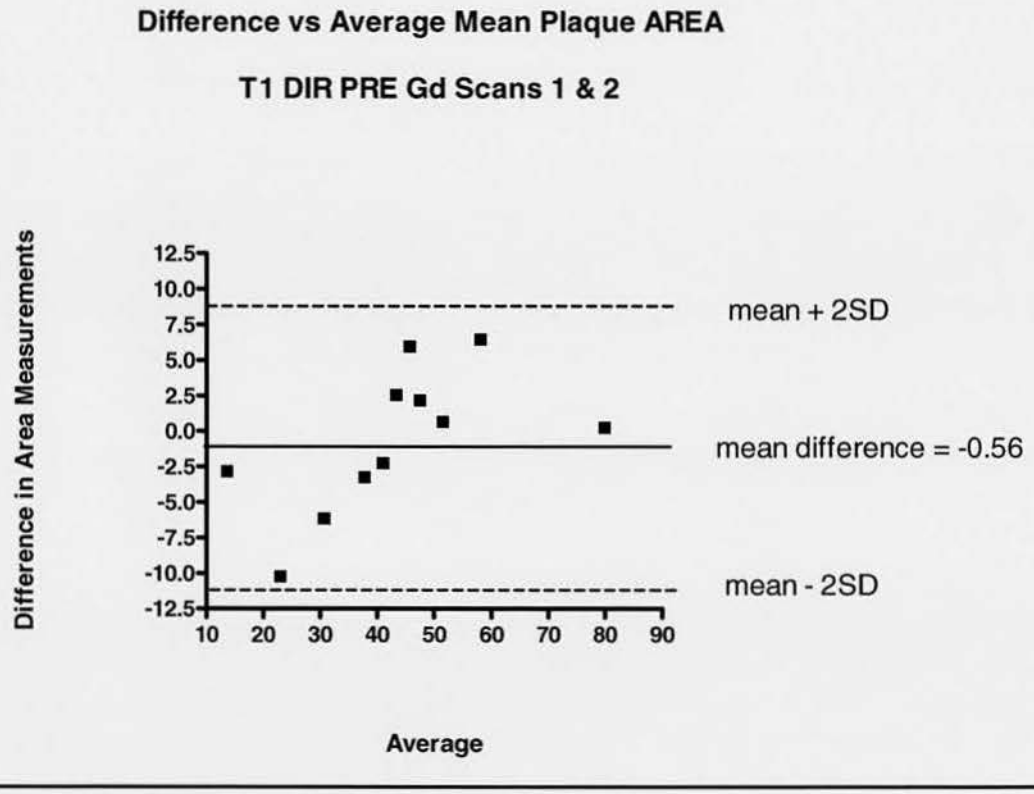

T1 Weighted
...the best quality.

Fig. 6.3


Difference vs Average Mean Plaque AREA
T1 DIR PRE Gd Scans 1 & 2
Difference in Area Measurements
12.5
10.0
7.5
5.0
2.5
0.0
-2.5
-5.0
-7.5
-10.0
-12.5
10
20
30
40
50
60
70
80
90
mean + 2SD
mean difference = -0.56
mean - 2SD
Average

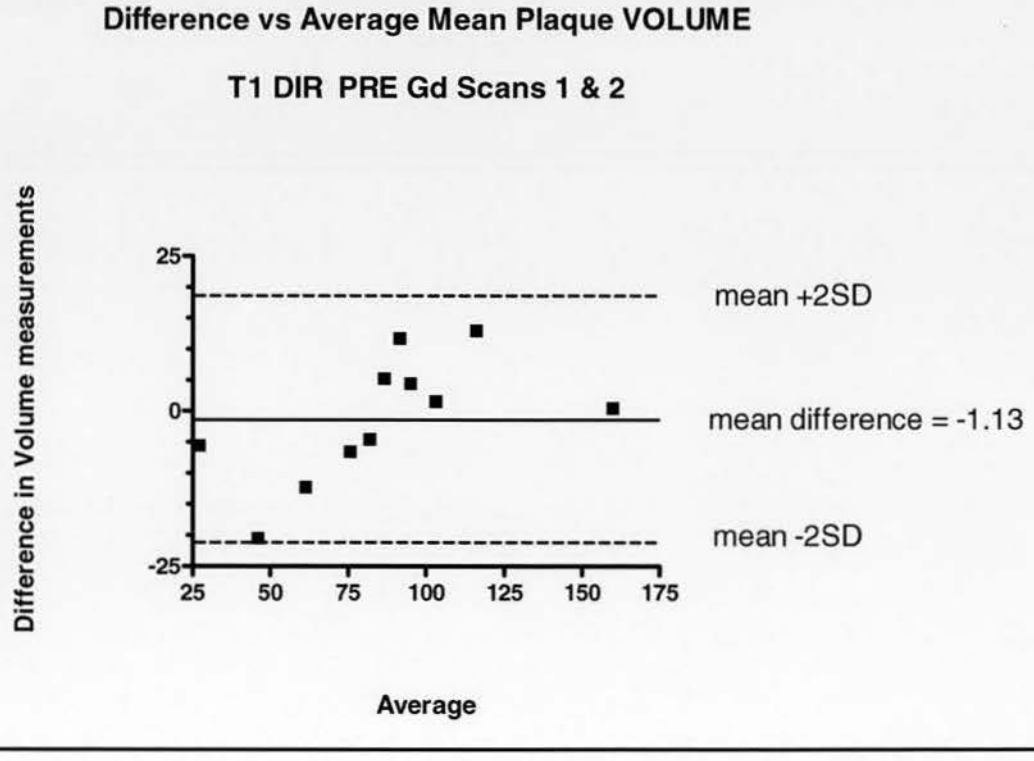
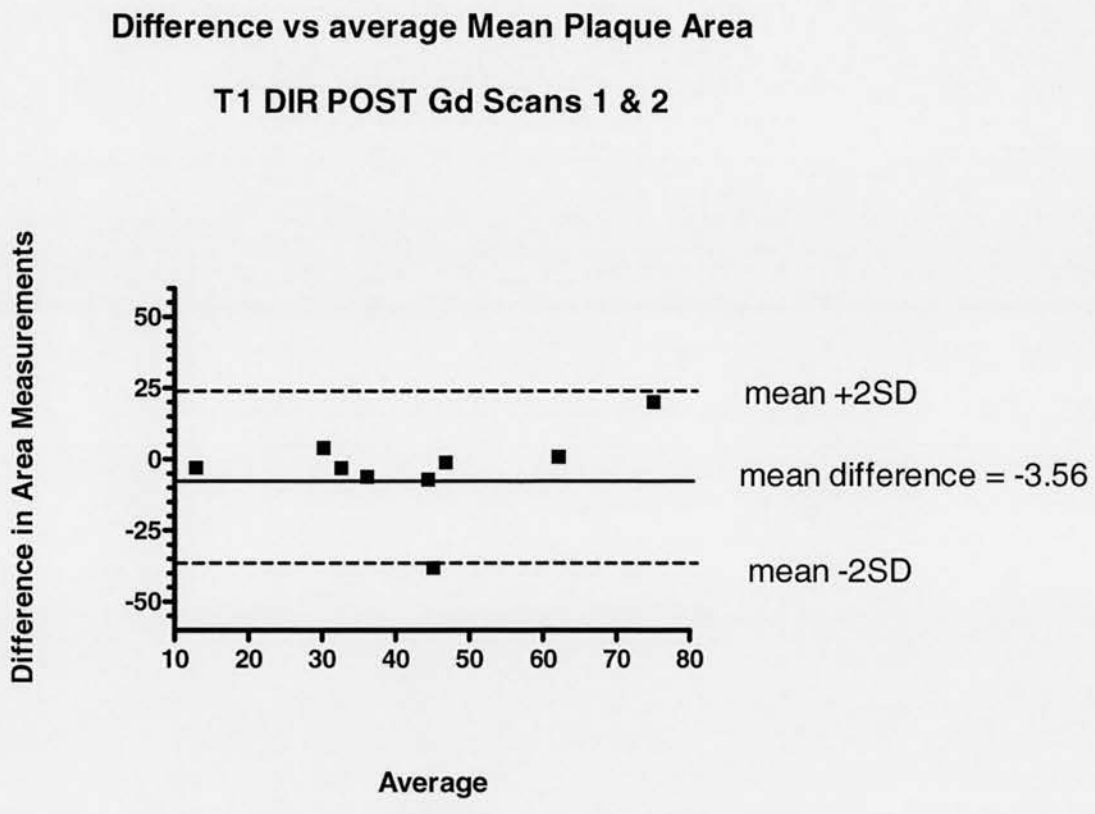

Difference vs Average Mean Plaque VOLUME
T1 DIR PRE Gd Scans 1 & 2
Difference in Volume measurements
25
0
-25
25
50
75
100
125
150
175
mean +2SD
mean difference = -1.13
mean -2SD
Average

Fig. 6.4


Difference vs average Mean Plaque Area
T1 DIR POST Gd Scans 1 & 2
Difference in Area Measurements
50
25
0
-25
-50
10
20
30
40
50
60
70
80
mean +2SD
mean difference = -3.56
mean -2SD
Average

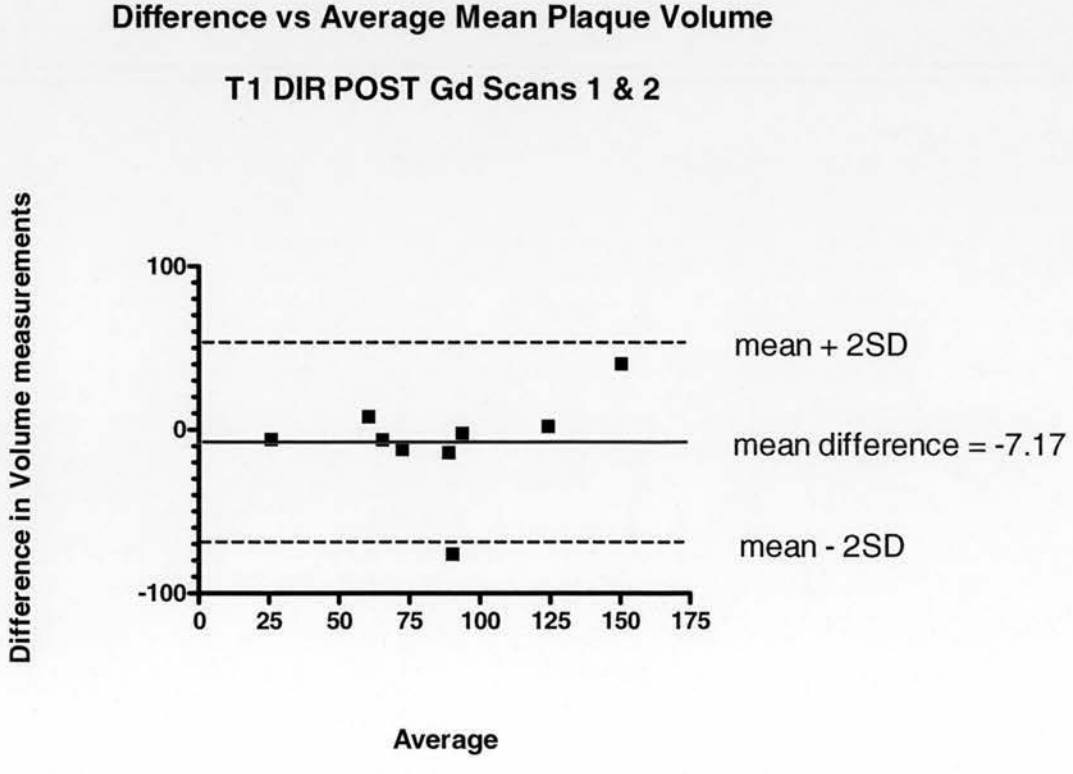

Difference vs Average Mean Plaque Volume
T1 DIR POST Gd Scans 1 & 2
Difference in Volume measurements
100
0
-100
0
25
50
75
100
125
150
175
mean + 2SD
mean difference = -7.17
mean - 2SD
Average

Fig 6.5 Histogram

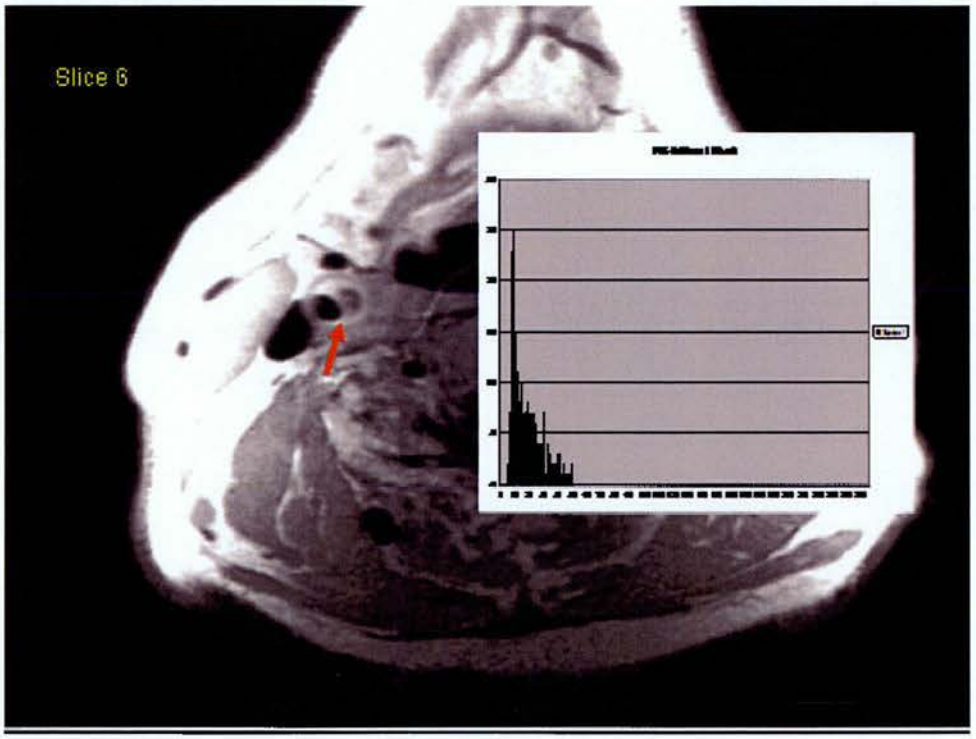


Fig. 6.6

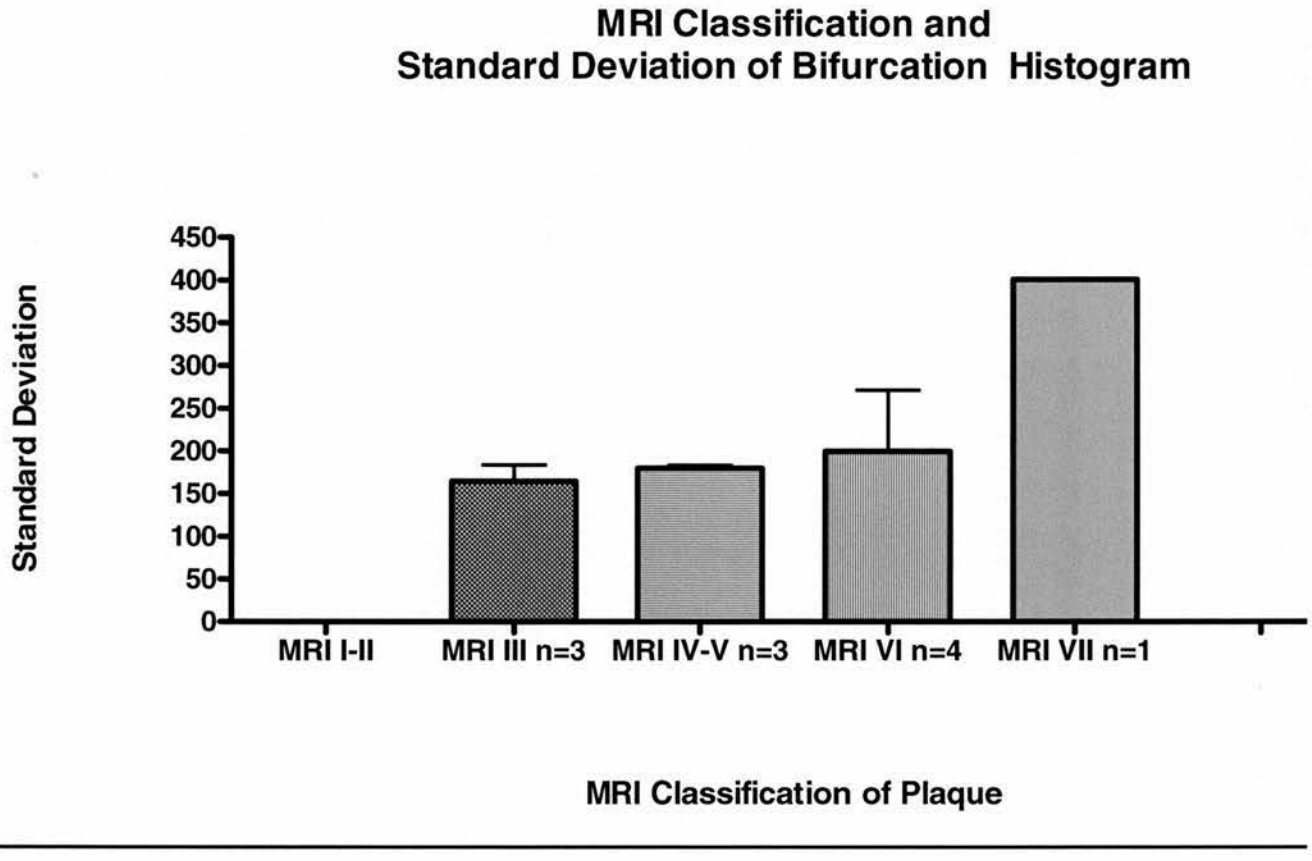

MRI Classification and
Standard Deviation of Bifurcation Histogram
Standard Deviation
450
400
350
300
250
200
150
100
50
0
MRI I-II
MRI III n=3
MRI IV-V n=3
MRI VI n=4
MRI VII n=1
MRI Classification of Plaque

Fig. 6.7 Signal intensity in atheromatous plaque and lumen of vein following intravenous gadolinium.

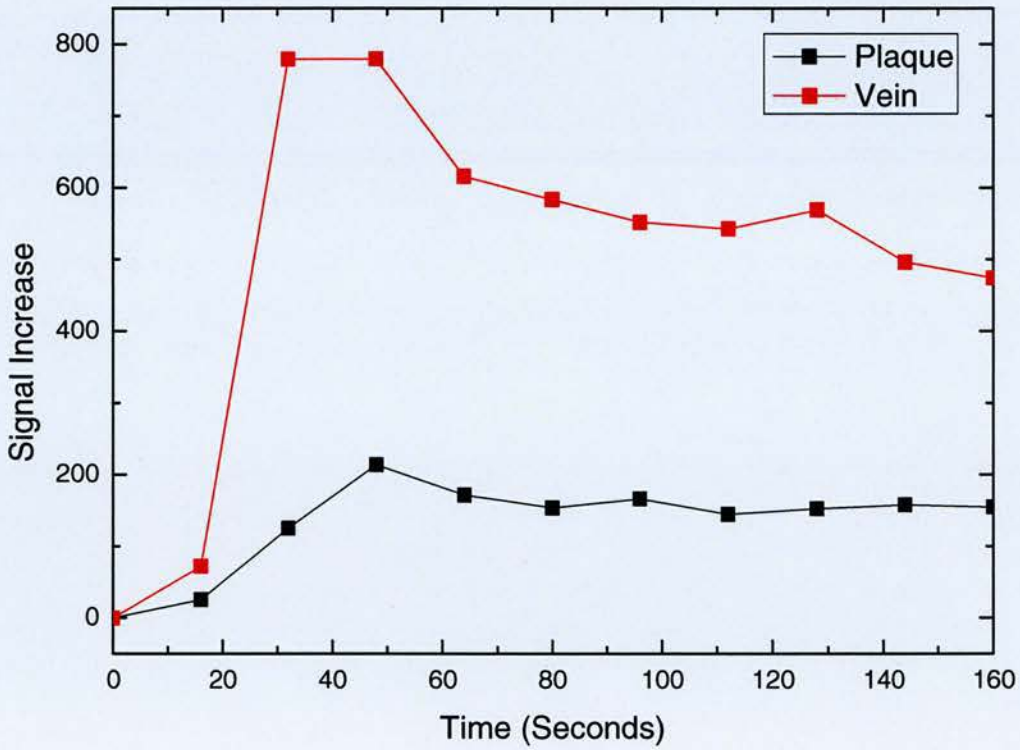


Fig. 6.8 Images of carotid artery plaque (yellow arrow), and jugular vein (green arrow); pre-contrast (1) and at subsequent time points following gadolinium injection (2-5).

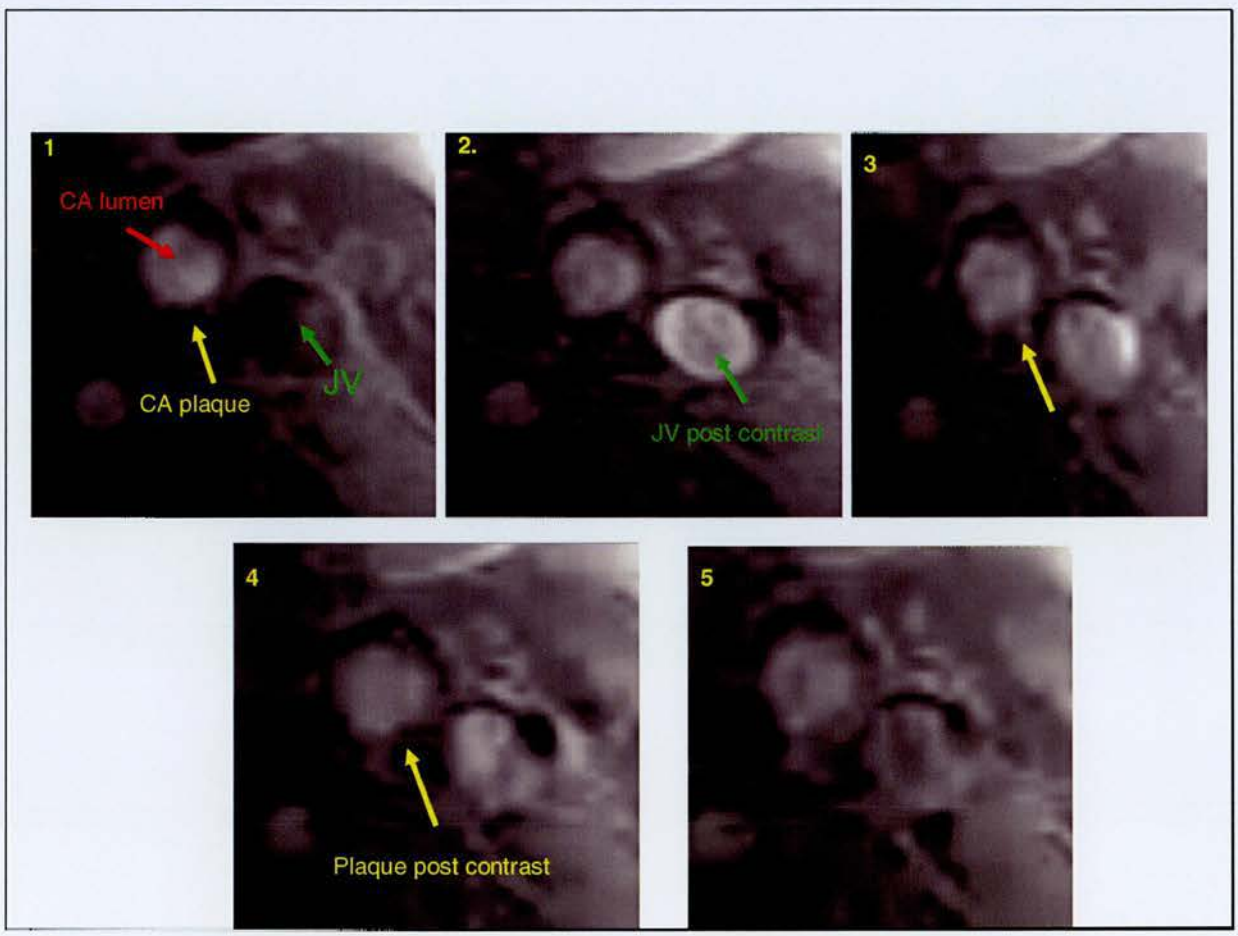
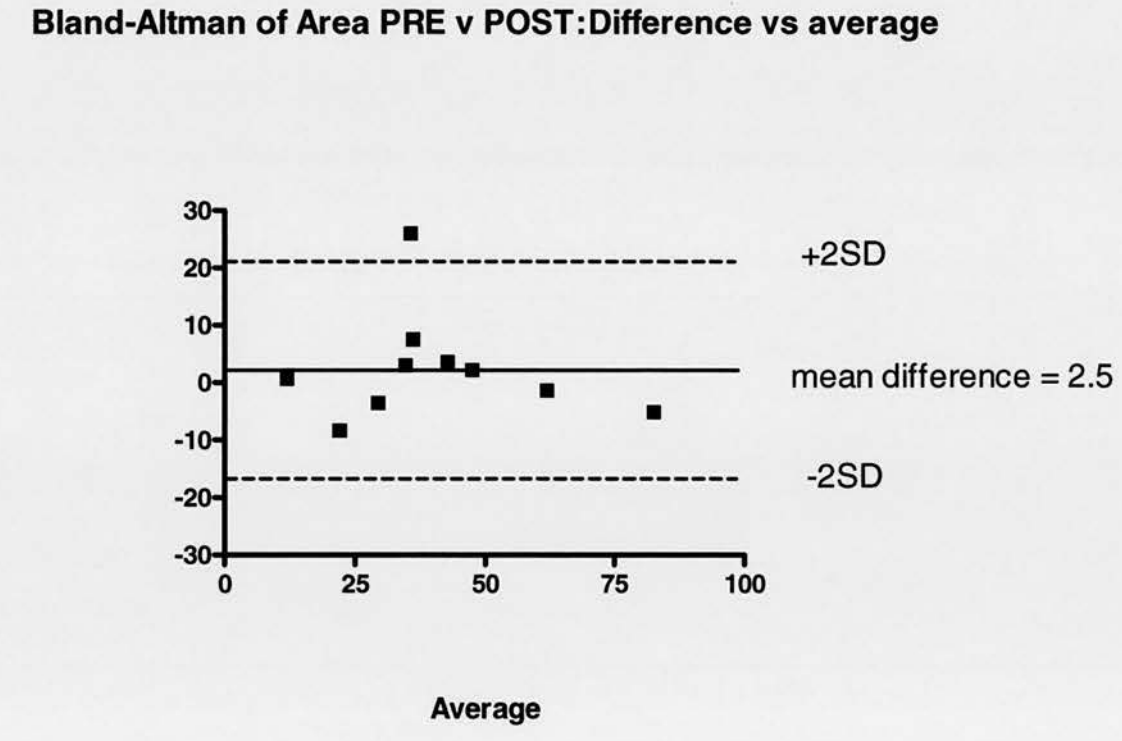


Fig. 6.9 Bland Altman plot looking at measurement of plaque area in pre- versus post-contrast enhanced T1-weighted scans



CHAPTER 7

SUMMARY AND FUTURE DIRECTIONS

In summarising the published data to date, along with the conclusions drawn from the body of research that comprises this MD thesis, it will be possible to comment upon the current status of non-interventional cardiac imaging and hypothesise as to where future research and innovations may lead.

7.1 CORONARY ARTERY CALCIUM SCORING

7.1.1 Method of Quantification

The correlation between coronary artery calcification and atherosclerotic disease has been documented since 1970 (Frink, Achor et al. 1970), but until more recently, there had been no specific method of quantifying the calcium load as detected by computed tomography. Agatston first described a scoring method in 1990 which has been the basis of the coronary calcium score since and upon which risk stratification has been based (Agatston, Janowitz et al. 1990). This scoring method is based upon the number, area and peak Hounsfield number of the calcific lesions identified, and is described in full in chapter 2. The accuracy and reproducibility of this measure, however, has been cast into doubt more recently, and alternative methods of quantification have been proposed and researched. Callister's group first proposed a volumetric score in 1998, and found it to have superior reproducibility when compared with the Agatston score (Callister, Cooil et al. 1998). This finding was repeated in 2002 by Kopp *et al*, who found that spiral MDCT with isotropic volumetric data improved reproducibility when compared with EBCT (Kopp, Ohnesorge et al. 2002). In the most recent and largest study to date, the Multi-Ethnic Study of Atherosclerosis (MESA) is a multicentre observational study of 6814 initiated to investigate the prevalence, correlates and progression of subclinical cardiovascular disease. Baseline measures were taken which included CT coronary

calcium score. Agatston scores, calcium volumes and interpolated volume scores were calculated. Volume scores are calculated using the technique of isotropic interpolation, whereby the input data reconstructed and sampled at arbitrary cross sections between the original cross section to correspond with pixel size. The smaller size of the interpolated voxel allows a more accurate volume reconstruction of the calcium volume when compared to using the original slice thickness. The interpolated voxels are assigned a numeric value and, as with the Agatston method, only those greater than a Hounsfield Unit value of 130 are used to represent calcified plaque. An automated 3D reconstruction is acquired and a final score calculated which corresponds to the calcium volume load. The actual value in cubic millimetres is multiplied by 1000 to generate whole numbers and facilitate comparison with the traditional calcium score. The MESA study concluded that it is possible to obtain similar reproducibility for coronary calcium scoring using either EBCT or MDCT and that, for either scanner, the volume scoring method results in only a minimal improvement in interscan reproducibility when compared with the Agatston score (Detrano, Anderson et al. 2005).

Overall, results from this study and other similar studies show that the calcium volume score is only slightly more reproducible when compared with the Agatston score (Callister, Cooil et al. 1998; Kopp, Ohnesorge et al. 2002; Ohnesorge, Flohr et al. 2002; Ulzheimer and Kalender 2003; Detrano, Anderson et al. 2005).

7.1.2 Screening

Much public funding is devoted to increasing the awareness of CHD and to identifying at risk populations in order to tackle primary prevention of the disease. A

widely use method of risk stratification is based on the Framingham study and takes into account age, total cholesterol, LDL cholesterol, systolic blood pressure, treatment for hypertension, and cigarette smoking. The Framingham risk score gives estimates for 'hard CHD' which includes myocardial infarction and coronary death. However, it cannot definitively give an answer as to the presence or absence of coronary disease. Coronary angiography can give information as to flow limiting lesions, but is an invasive procedure and identifies disease relatively late on. In addition, it is known that culprit lesions are often not flow limiting but are small (<70%) so-called vulnerable plaques. Computed tomography is a relatively safe, fast and accessible mean of measuring the coronary calcium score as a surrogate for atherosclerotic disease.

In order for a screening tool to serve its purpose, it must have a high negative predictive value. Shemesh *et al* have shown that, in asymptomatic patients, the absence of coronary calcification on CT is predictive of angiographically normal coronary arteries (Shemesh, Tenenbaum et al. 1996). Retrospective analyses have also suggested screening to be of benefit in asymptomatic patients with risk factors for CHD, although this appears to be less useful in individuals with fewer risk factors (Moser, O'Keefe et al. 2003). Until recently, there have been no large prospective studies to base recommendations upon. The Prospective Army Coronary Calcium (PACC) project, recruited 2000 healthy volunteers who were evaluated in terms of standard risk factors and CAC as measured by EBCT. The patients were followed up for a mean of three years in terms of subsequent coronary syndromes and sudden cardiac death. This study concludes that, in young asymptomatic men, coronary calcium scoring can provide a considerable, cost-effective, independent prognostic

indicator in predicting subsequent CHD event over and above current coronary risk factors.

Having established that CAC correlates with the risk of CHD events, it is important to determine a scale of risk stratification in order for clinicians to act upon the results. A recent metanalysis of studies that have attempted to determine a risk stratification model has sought to assess the predictive value of the coronary calcium score after adjustment for standard CHD risk factors. The meta-analysis found a high degree of variation in the relative risk associated with different CAC scores, which appears to be due to many factors including method of quantification, trial conduct and the proportion of women recruited. It concluded that the coronary calcium score is an independent risk factor for CHD events and that even people with a low score (1-100) have around twice the risk of CHD events, with high scores (>400) having a risk of anywhere between 4.3 – 17 times the risk (Pletcher, Tice et al. 2004). More recently, the St. Francis Heart Study recruited 1,005 participants with a baseline coronary calcium score above the 80th percentile for age, who were randomised to treatment with atorvastatin, vitamin C and vitamin E (alpha-tocopherol) versus aspirin and matching placebo. The relationship of events with baseline calcium score and standard risk factors was assessed; it was found that the baseline score was higher in those who suffered subsequent CHD events and that there was a greater increase in coronary calcium score in those with subsequent CHD events. Change in coronary calcium score after adjustment for standard CHD variables, however, did not predict events (Arad, Goodman et al. 2005). The study did not publish data on relative risk associated with extent of CAC. Most recently, data from the MESA study demonstrated that CAC may be used in intermediate risk patients to identify those

who are at higher risk. However, it highlighted the considerable gender and ethnic variations which make setting specific cut off points for risk hard to set, thereby limiting its use as a screening test. The MESA study co-ordinators highlight the necessity for further evaluation of risk as well as potential cost benefit of using the presence of CAC as a screening tool in 'at-risk' patients (Lakoski, Cushman et al.).

To summarise, it is clear that there is an association between the extent of CAC present and subsequent risk of CHD events. As yet however, there are no specific guidelines regarding relative risk and threshold for treatment. In 2000, the ACC/AHA Expert Consensus Document on electron-beam computed tomography for the diagnosis and prognosis of coronary artery disease concluded that the absence of CAC is a good negative prognostic indicator and that a positive test confirms the presence of atherosclerotic plaque. It also concludes that the greater the coronary calcium score, the greater the likelihood of prognostic disease; the relationship, however, is not linear and the true plaque burden may be underestimated (O'Rourke, Brundage et al. 2000). Since then, a number of larger scale studies have been commenced, including the St. Francis Heart Study and the PACC project which have recently been completed. Due for completion in the near future; the Risk Factors, Evaluation of Coronary Calcium and Lifestyle (RECALL) study, a population based prospective cohort study of the comparative value of risk stratification techniques for cardiac events. This study is powered to define the relative risk associated with the extent of coronary atherosclerosis as measured by the coronary calcium score. It aims to identify at risk groups who are most likely to benefit from primary prevention (Schmermund, Mohlenkamp et al. 2002).

Currently, the coronary calcium score is looked upon as a new and very exciting method of detecting subclinical atherosclerosis and many centres offer this as a screening tool. However, there are as yet no specific evidence based guidelines as to the precise implications of the result, and no guidelines as how best to manage patients based upon their result. In the near future, with the completion of large population based studies, it is likely that we will have a better idea of the exact implications and usefulness of the coronary calcium score as a screening tool.

7.1.3 Coronary Artery Calcification Progression and Risk of Subsequent Coronary Heart Disease Events

The coronary calcium score has been used to track changes in the coronary atherosclerosis non-invasively. In 2000, Shemesh *et al* reported that EBCT can be used to track changes in coronary calcium score over time, as a useful way of tracking changes in coronary atherosclerosis (Shemesh, Apter et al. 2000). However, the study does not correlate this with clinical events and nor does it publish reproducibility data on their scoring method. Since then, an observational study of 817 patients who were referred for two sequential EBCT scans reported a greater increase in coronary calcium score in patients who had an MI compared to those who remained event free (Raggi, Cooil et al. 2003). The St. Francis Heart Study is the first large prospective study to draw conclusions on the significance of progression of CAC. It recruited 1,005 subjects with a coronary calcium score of >80th percentile for age and gender and found a greater increase in baseline score in patients who subsequently experienced CHD events than in those who remained event free (Arad, Spadaro et al. 2005).

Whilst there is a body of evidence to support progression of CAC as an indicator of subsequent risk of CHD events, there are no data to support the routine practice of serial CT scans to track disease progression. For example, how frequently would the scans require to be performed to notice a trend in change and, if it were an advocated method of follow up in such patients, would this be done for life? Furthermore, how would this actually affect patient management? Would a perceived positive response to treatment result in cutting back in, or alteration to the treatment regimen? Not only would this place an enormous burden on healthcare funding and resources, but there is a significant radiation dose incurred with repeated CT. Such exposure to ionising radiation is impossible to justify without a clear evidence basis supporting the investigation in question, nor indeed as to how to interpret the results and base treatment upon thereafter.

7.1.4 Coronary Artery Calcification as a Measure of Response to Therapy

The recent publication of the St. Francis Heart Study and the Beyond Endorsed lipid Lowering with EBT Scanning (BELLES) study has concluded many years of controversy over the role of serial coronary calcium scoring in monitoring disease response to therapy. Callister's group, in 1998, conducted a retrospective study of patients who had undergone two screening EBCT scans more than a year apart, and found that the extent of progression, stabilisation or regression was directly related to HMG CoA reductase inhibitors (Callister, Raggi et al. 1998). Budoff *et al* in 2000 also documented a reduction in progression related to statin therapy in a retrospective observational study (Budoff, Lane et al. 2000). In 2002, Achenbach *et al* conducted an uncontrolled prospective evaluation study which indicated that cerivastatin reduces progression of CAC (Achenbach, Ropers et al. 2002). In contrast to these findings, in

2003, Hecht *et al* published results of a non blinded comparative observation study of more versus less aggressive lipid-lowering therapy. They found that, whilst there was a significantly greater reduction in LDL-cholesterol levels, there was no change in the degree of CAC (Hecht and Harman 2003).

The striking feature of the studies described is that none is a prospective, blinded, placebo-controlled study and therefore the results have to be interpreted with extreme caution. Many patients have undergone serial CT scans to monitor therapy on the basis of the above studies which support the modality as a means of detecting response to therapy. A more definitive answer has been provided with the completion of the BELLES and St. Francis Heart Study.

BELLES randomised 615 hyperlipidaemic postmenopausal women to intensive versus moderate lipid lowering therapy (atorvastatin 80 mg and pravastin 40 mg respectively). Patients underwent EBCT at baseline and after one year of treatment. The study found no less progression of CAC in the aggressive lipid-lowering arm despite superior LDL-cholesterol lowering (Raggi, Davidson et al. 2005). The study is limited, however, by the relatively short period of follow-up and the absence of a placebo group. The St. Francis Heart Study randomised 1,005 patients with a coronary calcium score greater than the 80^{th} percentile for age either to treatment with atorvastatin 20 mg, vitamin C 1 g daily and vitamin E 1,000 U daily or to matched placebo for a mean treatment period of 4.3 years. They found that, whilst there was a significant reduction in LDL-cholesterol and also in CHD events in the treatment group, this did not affect the progression of CAC as measured by EBCT (Arad, Spadaro et al. 2005). In contrast to the BELLES study, the St. Francis Heart Study

was placebo controlled and participants followed up for over 4 years. This study is pivotal in defining the role of coronary calcium scoring in current clinical practice.

When the ACC/AHA Expert Consensus Document on electron-beam computed tomography for the diagnosis and prognosis of coronary artery disease was published in 2000, it identified grey areas, or deficiencies in the then current body of evidence. First of all, it concluded that the literature showed that EBCT, whilst having a high sensitivity, had a low specificity and overall predictive accuracy of around 70%. It was felt that EBCT did not provide a superior alternative to existing non-invasive diagnostic methods such as SPECT imaging. The document stated that, in order to be of value, the test should confer an added prognostic benefit over and above readily available methods such as the Framingham risk model, described earlier. Furthermore, at the time, the published literature was not able to define specific cohorts who would benefit from EBCT and stressed that the screening modality should not be readily available to the public without specific referral from a physician. Another area which was highlighted was the inter-observer and inter-test variability. The document concluded that further properly designed outcomes research was needed, along with studies assessing the role of serial EBCT scanning in progression or regression of CHD.

Since 2000, the studies published and summarised above, namely the St. Francis Heart Study, BELLES, MESA and PACC go a long way to providing answers to the above questions. Due for completion in the near future, RECALL should provide the additional evidence with which to formulate guidelines as to who is likely to benefit from CAC screening.

7.1.5 Summary of Thesis Research Conclusions

We have confirmed that, despite marked reductions in serum LDL cholesterol and C-reactive protein concentrations, atorvastatin 80 mg daily did not halt the progression, or induce regression, of coronary artery calcification in patients with calcific aortic stenosis. Consistent with recent trials of asymptomatic individuals (Arad, Spadaro et al. 2005; Raggi, Davidson et al. 2005), our findings are in marked contrast to previous observational studies and suggest that the potential beneficial effects on coronary artery calcification have been over estimated. We have demonstrated good reproducibility of coronary artery calcification scores in patients with scores of greater than 100 AU. In contrast to the BELLES and St. Francis Heart Study, our cohort was selected upon the basis of the presence of calcific aortic stenosis, and was treated only with statin, rather than statins and vitamin E. However, the fact that we reached the same conclusions further supports the fact that, whatever the population, statins do not reduce CAC.

With the constant development of newer and more sophisticated technology, in particular MDCT, the accuracy and reproducibility of the modality continues to improve. This will enable the coronary calcium score to be used more predictably for defining risk of future cardiac events. With increasing experience and use of the imaging modality, if differences in the coronary calcium score over time are proven to result in different event rates then it is likely that serial measurements will provide a powerful predictive tool enabling treatment to be tailored to each individual. We conclude that intensive lipid lowering therapy does not halt the progression, or induce regression, of coronary artery calcification. Although coronary artery calcium scores

correlate well with the presence of atherosclerosis and predict future coronary risk, our findings confirm that there is no role for monitoring progression of coronary artery calcification in order to assess the response to lipid lowering therapy.

7.2 COMPUTED TOMOGRAPHY ANGIOGRAPHY

7.2.1 Technology

The advantage that invasive coronary angiography, the current gold standard method of imaging coronary vessels, holds over computed tomography, is its excellent temporal resolution (<10ms) This is of importance due to the fact that imaging of the heart must be performed during fast and complex cyclical motion with considerable beat to beat variation. The temporal resolution of even the most technologically advanced CT scanner is significantly poorer than this, at between 50-300 ms. It is possible to minimise motion artefacts by acquiring images during late diastole when there is little cardiac motion.

The disadvantage of coronary angiography is that it is an invasive procedure which is associated with the risk of stroke, myocardial infarction and death as well as the risk of local complications at the puncture site. Furthermore, while it provides precise information on lumen diameter and the presence and site of stenosis, invasive coronary angiography provides no information on the state of the vessel wall. For example, it is known that luminal stenosis is a relatively late feature in the natural history of atherosclerosis, with positive arterial remodelling acting to compensate for the development of atherosclerotic plaque. Thus, a normal vessel lumen size at angiography may grossly underestimate the extent of disease. Intravascular

ultrasound has the ability to provide information as to vessel wall properties and atherosclerotic plaque volume but, again, is an invasive procedure.

7.2.2 CT Angiography – Native Vessels

In carefully selected patients, CT angiography is becoming a safe, feasible and accurate means of detecting native coronary vessel patency and the presence of significant stenoses. A recent meta-analysis reviewed the literature from 1950 until March 2005 and drew conclusions as to the sensitivity and specificity of contrast-enhanced MDCT for the detection of coronary artery disease (Stein, Beemath et al. 2006). To summarise, they found that there was at least 95% sensitivity for the detection of significant stenoses, namely stenoses of ≥50%, with 4-, 16-, and 64-slice MDCT. As would be expected, the specificity increased with the number of detectors, as did the sensitivity for significant stenoses in evaluable segments and the overall number of evaluable segments. They found that the published data reported a higher sensitivity for stenoses in proximal and mid segments as opposed to distal segments. When evaluating lesions in named arteries, the sensitivity was higher for the left main stem and left anterior descending arteries than for the circumflex and right coronary arteries, although the differences were small with the latest 64-slice scanner. A summary of the literature is shown in Table 1.

Table 7.1. Detection of significant stenosis (≥50%) according to coronary artery segment

	Year published	No. of detector rows	No. of patients	Sensitivity (%)	Specificity (%)
Neiman *et al(Nieman, Rensing et al. 2002)*	01	4	53	82	93
Nieman *et al(Nieman, Oudkerk et al. 2001)*	01	4	31	81	97
Becker(Becker, Knez et al. 2002)	02	4	28	81	90
Kopp(Kopp, Schroeder et al. 2002)	02	4	102	86	96
Leber *et al(Leber, Knez et al. 2003)*	03	4	91	82	96
Gaudio *et al(Gaudio, Mirabelli et al. 2005)*	05	4	61	77	91
Dirksen(Dirksen, Jukema et al. 2005)	05	4	25	95	91
Maruyama(Maruyama, Yoshizumi et al. 2004)	04	8	25	90	99
Matsuo(Herzog, Ay et al. 2001)	04	8	25	75	96
Neiman(Nieman, Cademartiri et al. 2002)	02	16	58	95	86
Mollet(Mollet, Cademartiri et al. 2004)	04	16	127	92	95
Kuettner(Kuettner, Trabold et al. 2004)	04	16	58	72	97
Leta(Leta, Carreras et al. 2004)	04	16	31	75	91
Hoffman U(Hoffmann, Moselewski et al. 2004)	04	16	33	63	96
Martuscelli(Martuscelli, Romagnoli et al. 2004)	04	16	61	89	98

Paul(Paul, Ohanessian et al. 2004)	04	16	29	86	99
Candemartiri(Cademartiri, Runza et al.)	05	16	40	96	96
Schuijf(Schuijf, Bax et al. 2005)	05	16	45	98	97
Mollet(Mollet, Cademartiri et al. 2005)	05	16	51	95	98
Kuettner(Kuettner, Beck et al. 2005)	05	16	72	82	98
Hoffman MHK(Hoffmann, Shi et al. 2005)	05	16	102	95	98
Leschka(Leschka, Alkadhi et al. 2005)	05	64	67	94	97

As suggested above, MDCT angiography has its limitations. The image quality is degraded by artefacts in patients with irregular hear rhythms and breathing during the scan. Large calcific deposits in atherosclerotic plaque cause beam hardening and partial volume artefacts which can completely obscure the cross section of the vessel and prevent assessment of patency. Similarly, metal objects such as stents, surgical clips and sternal wires can interfere with image quality. The right coronary artery is the most rapidly moving, especially in its mid portion, and motion artefacts are most frequently seen there. The motion pattern of the left anterior descending and circumflex arteries are related to left ventricular contraction, whereas the right coronary artery moves synchronously with the right side of the heart. This causes problems with reconstruction algorithms. The optimal phase for viewing the left sided arteries may be suboptimal to view the right coronary artery. However, with the improved temporal resolution provided by 64-slice CT, and ever improving

reconstruction algorithms, these factors which degrade the image quality can be minimised. Whilst with older 4-slice CT scanners, only 78% of segments could be evaluated, 91% could be evaluated with 16-slice and all segments with 64 slice (Stein, Beemath et al. 2006). Recent data show that the 64 slice CT provides high diagnostic accuracy in assessing coronary artery stenoses and, importantly, when compared to older generation scanners, has ability to assess a greater proportion of coronary artery segments (Leschka, Alkadhi et al. 2005). With the advent of 128-slice scanners, this accuracy would be expected to improve further and has the potential of providing an accurate and non-invasive means of assessing coronary artery stenoses.

7.2.3 CT Angiography – Coronary Artery Bypass Grafts

Even in its early stages, computed tomography has been investigated as a means of non-invasively determining the patency of coronary artery bypass grafts. Coronary bypass grafts are more easily imaged than native vessels due to their large calibre (in the case of venous grafts) and reduced susceptibility to cardiac motion artefacts. Sensitivities and specificities of greater than 90% were achieved even with single slice spiral CT (Engelmann, von Smekal et al. 1997). The data regarding graft patency has been discussed extensively in Chapter 5; Table 4, Chapter 5 provides pooled data regarding the sensitivity and specificity of MDCT in determining graft patency in the literature. Data on the accuracy of CT for the detection and grading of haemodynamically significant stenoses, however, is relatively lacking and tend to be based on small study population size. Nonetheless, CT angiography with 16- slice CT allows accurate assessment of venous and arterial grafts in patients who have no contra-indications to MDCT angiography.

7.2.4 Summary Of Thesis Research Conclusions

MDCT provides a highly specific means of detecting coronary artery bypass graft patency in a clinical setting. It is safe and abolishes the need for time consuming invasive coronary angiography which is associated with the small but significant risk of major complications. In contrast to invasive coronary angiography, it can be performed on a scheduled out-patient basis rather than requiring admission to a day case unit. In line with recent studies, we can confirm that MDCT is highly specific for the detection of bypass graft patency. It provides a safe, fast and cost effective means of imaging coronary artery bypass grafts but with the disadvantage of a high radiation dose. The introduction of 64 slice CT scanners will result in further improved image quality which may enable non-invasive imaging to be the mainstay of assessment of graft patency in the future.

7.3 MAGNETIC RESONANCE ANGIOGRAPHY

MR angiography with intravenous contrast has become a routine method of assessing the degree of stenosis of the carotid, aorta, renal and peripheral arteries. MRA techniques have also been proposed for assessment of the coronary vasculature. I propose to focus on the literature examining the use of MRA in the carotid vessels.

7.3.1 Lumen Imaging

The carotid artery is primarily imaged using 2D time of flight (TOF), 3D TOF and contrast enhanced MRA. 2D tends to offer the best edge definition but is susceptible to artefact from patient movement. Three dimensional techniques, however, give better spatial resolution and create fewer artefacts. The use of gadolinium as a contrast agent acts to increase the signal of blood in relation to surrounding tissue, and

reduces the time required for image acquisition (10-20 seconds *vs.* around 10 mins with standard TOF acquisition). However, in comparison to TOF, contrast-enhanced MRA (CE-MRA) has been shown to increase the demonstrated degree of stenosis by an average of 20-30% (Townsend, Saloner et al. 2003). In order to determine stenosis severity, therefore, only axial TOF images should be used.

Various papers have investigated the correlation between stenosis severity as assessed by MRA versus ultrasound and digital subtraction angiography (DSA). Patel *et al* found that MRA correlated well with ultrasound, with 3D TOF outperforming 2D in terms of sensitivity and specificity when single imaging modalities were compared to angiography (Patel, Kuntz et al. 1995). Anderson *et al* reported MRA and DSA to be similar in their assessment of carotid bifurcation stenosis, with 2D and 3D TOF MRA comparing more closely with DSA than ultrasound (Anderson, Saloner et al. 1992). Both authors concluded that DSA is only required to clarify stenosis severity when ultrasound and MRA are discordant. This method is safer, as it avoids the risks associated with an invasive procedure, and is also more cost effective (Anderson, Saloner et al. 1992).

7.3.2 Plaque Imaging

The three most important aspects of plaque morphology in atherosclerosis imaging are (i) plaque size, thickness, eccentricity and distribution along the vascular bed; (ii) plaque composition with respect to lipid/necrotic core, fibrous matrix, haemorrhage and calcification and (iii) plaque inflammation. The various properties of these components have been characterised in terms of their signal intensity characteristics on T1-, T2-, and intermediate-weighted images and have been described in Chapter 1.

In recent years, various centres have investigated the validity of these measures. Misumori *et al* compared measurements of vessel wall volume, maximum wall area and minimum lumen area determined using *in vivo* black blood MRI assessment and *ex vivo* in patients undergoing carotid endarterectomy. Results indicate that *in vivo* black blood MRI can provide accurate measurements on different aspects of carotid atherosclerotic plaque burden (Mitsumori, Hatsukami et al. 2003). The technique has also been found to be reproducible (Kang, Polissar et al. 2000). In terms of plaque composition, MR has been used to identify unstable plaque (Hatsukami, Ross et al. 2000; Mitsumori, Hatsukami et al. 2003; Trivedi, J et al. 2004), soft plaque (necrotic core or haemorrhage) (Yuan, Mitsumori et al. 2001) and intraplaque haemorrhage (Chu, Kampschulte et al. 2004) with high levels of agreement with histological findings. This information has enabled classification of human carotid atherosclerotic plaques according to American Heart Association (AHA) classifications. A recent study of 60 patients who underwent MR scanning prior to carotid endarterectomy showed good agreement between MR imaging and AHA classification (Cai, Hatsukami et al. 2002).

7.3.3 Monitoring of Therapy

Imaging in clinical trials is required to be non- or minimally invasive, to provide quantitative information on plaque morphology, tissue components and inflammatory activity of lesions at various stages and be fully validated against a pathological gold standard. In addition, they should allow repeated study of the same vascular bed, using reliable landmarks in a multicentre environment where small changes can be identified consistently. Validation studies to support such requirements are summarised above and provide the basis for several past and ongoing trials. Zhao *et*

al have demonstrated a reduction in plaque lipid volume, as assessed by MRI, with prolonged lipid lowering therapy (Zhao, Yuan et al. 2001). Corti *et al* demonstrated that maintained lipid-lowering therapy is associated with regression of atherosclerotic lesions and is associated with sustained vascular remodelling (Corti, Fuster et al. 2002). Whilst these studies were based in a single centre, promising results as to the feasibility of mulicentre trials are available. Chu *et al* acquired quantitative measurement of lumen, wall, outer wall, lipid-rich/necrotic core and calcification with excellent correlation across four time points at five different clinical sites (Chu, Kampschulte et al. 2004). ORION (Outcome of Rosuvastatin treatment on carotid artery atheroma: a magnetic resonance Imaging ObservatioN) is an ongoing study to assess the effect of lipid lowering on progression of atherosclerosis using multicontrast, high-resolution MRI. The study has reported that MRI can reproducibly determine lesion types and the distribution of lesion in hypercholesterolaemic patients with moderate carotid artery stenosis (Chu, Hatsukami et al. 2004).

7.3.4 Summary of thesis research conclusions

We have shown that it is feasible to recruit patients with recent acute carotid plaque events for MRI scanning within an acceptable time frame. MR imaging of the carotid bifurcation using T1 weighted sequences has been used to provide statistically significant, reproducible information, similar to that reported in the literature. The tendency of contrast agent to increase the variability of measurements has been suggested, although this was not statistically significant. MRI features have been used to grade plaque morphology allowing correlation of plaque heterogeneity with signal intensity heterogeneity as demonstrated by plotting histograms. Finally, we

have been able to conduct a protocol for the demonstration of plaque neovascularisation, reproducing previously reported results.

7.4 FUTURE DIRECTIONS

7.4.1 Coronary Artery Calcium Scoring

As discussed above, data published as part of this body of research along with international data has shown that there is no rationale for the use of serial coronary calcium scoring in order to monitor response to lipid-lowering therapy. However, there is evidence to support its use as a diagnostic tool to aid in the treatment and prevention of cardiovascular diseases, as its presence is indicative of the presence of underlying atheromatous disease. Screening of high risk populations, or those for whom conventional first line investigations have been equivocal, is a real possibility in the near future.

7.4.2 Therapy Targeted At Lowering Coronary Artery Calcification

Whilst lipid-lowering has proven benefits in the treatment of atherosclerosis, it has no effect on the coronary calcium score. Progression of CAC is associated with an increase in cardiovascular events (Raggi, Cooil et al. 2003; Arad, Spadaro et al. 2005) and therefore it would follow that therapy aimed at reducing CAC would be beneficial. The pathogenesis of atherosclerosis has been discussed in Chapter 1, but is still not fully understood. Recent reports have suggested an infectious aetiology, with infectious blood nanobacteria thought to have a role in triggering the process (Puskas, Tiszlavicz et al. 2005). Maniscalco *et al* found that combined EDTA and tetracycline therapy resulted in significant regression of calcified coronary plaque volume of, on average 14%. This was associated with a decrease in anginal

symptoms, although it was not powered to detect a change in event rate. This study, whilst small numbers and a relatively short treatment period (four months), is promising; larger placebo controlled studies are required to assess possible future therapy.

Previous studies have demonstrated an association between a deletion polymorphism of the angiotensin converting enzyme (ACE) gene and an increased risk of coronary artery disease (Nakai, Itoh et al. 1994) and myocardial infarction (Cambien, Poirier et al. 1992). Furthermore, the incidence and extent of CAC is directly related to the polymorphism; the ACE DD (homozygous for the ACE deletion) genotype is an independent determinant of CAC (Pfohl, Athanasiadis et al. 1998). Recently, a retrospective study, which assessed the rate of change of aortic valve calcification as measured by EBCT, observed a significant reduction in the rate of aortic valve calcium accumulation in patients who were receiving ACE-inhibitor therapy (O'Brien, Probstfield et al. 2005). A link between pathogenesis of aortic valve calcification and arterial atherosclerotic plaque has been suggested (Mohler III 2000) and, therefore, it may be extrapolated that ACE-inhibitors may act also to reduce the extent of CAC.

7.4.3 Plaque Imaging

Whilst CAC score can predict those at risk of cardiovascular disease/events, it gives no information on plaque morphology. MR techniques have been described which give information as to plaque volume, composition and fibrous cap using standard sequences with and without the addition of standard MR contrast agents. However, a contrast agent used to identify macrophages in patients with lymph node metastases was noted incidentally to produce signals in aortic plaques (Schmitz, Taupitz et al.

2001). This contrast agent consists of ultrasmall superparamagnetic particles of iron oxide (USPIOs), is available for clinical use (Sinerem®, Guerbet Laboratories), and is a potential method of looking at monocyte and macrophage activity in atherosclerotic plaques; a fundamentally important process in atherogenesis and plaque remodeling. Animal studies suggest that this is a promising technique to observe macrophage traffic in hyperlipidaemic model (Ruehm, Corot et al. 2001). In patients given Sinerem 24 hours before carotid endartectomy (Kooi, Cappendijk et al. 2003), histology has confirmed localisation of USPIOs to the atherosclerotic plaque. Recently, the novel contrast agent has been used to identify plaque inflammation in patients with symptomatic carotid stenosis by demonstrating accumulation of USPIO in macrophages within the plaques(Tang, Howarth et al. 2006; Trivedi, Mallawarachi et al. 2006). Currently, this contrast agent is not yet licenced for use in MRA, but phase III clinical trials are underway. This would provide yet more information as to plaque vulnerability and help target therapeutic agents.

7.4.4 MR Spectroscopy

MR spectroscopy (MRS) is used to characterise biochemical components in tissues of interest by using frequency information, instead of to provide spatial or positional information as in MRI, to identify different chemical compounds. Initial data published in 1990 showed that it could be used to distinguish mobile lipids and differential between normal triglyceride content and cholesterol-enriched lipids in animal models (Booth, Honey et al. 1990). More recently, image-guided MRS has been shown accurately to identify cholesterol ester in discrete pre-selected regions of atherosclerotic plaque as small as 1 mm^3 in ex vivo studies (Ruberg, Viereck et al. 2006).

Magnetic resonance spectroscopy can also be used to estimate tissue temperature. In ischaemic stroke, our centre has recently shown that the temperature in an acute lesion is higher than normal brain (Figure 7.1). This may reflect a pro-inflammatory response, and be linked to the pyrexia observed in those patients who have a worse outcome. Magnetic resonance spectroscopy has the potential to be developed to assess local tissue temperature changes in association with ischaemia or inflammation both in the brain and other tissues including atherosclerotic plaques. This would be complemented by tracking inflammatory cells using USPIO and SPIO imaging techniques as described above.

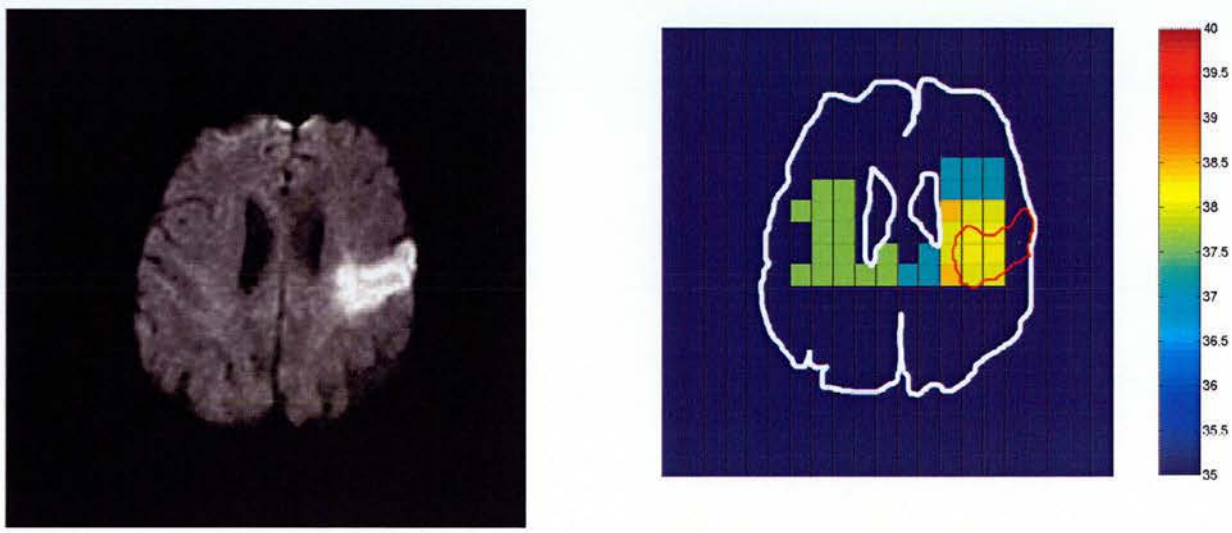


Figure 7.1

Ischaemic stroke imaged by magnetic resonance diffusion weighted image (left) and spectroscopy (right). The hottest areas (reds and yellows) are in the infarct itself and in the immediate surrounding tissue – the ischaemic penumbra.

7.5 Conclusion

We have established, according to the original aims set out in Chapter 1, that computed tomography can measure coronary calcium score in a reproducible manner, and that lipid-lowering has no effect on the progression of coronary calcification. We have established that MDCT can be used reliably to predict graft patency in patients who have undergone coronary artery bypass grafting. Finally, we have pilot data to support the role of MRI to assess plaque volumes and characteristics.

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