Non-Invasive Imaging of Atherosclerotic Plaque
Prior to Percutaneous Interventional Procedures

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Atherosclerosis is a disease which has impacted our health like no other in the last half century. The detection of this disease range from biomarkers, stress-testing to invasive imaging by way of angiography or other intravascular methods. In recent years, technological developments in multi-detector computed tomography (MDCT) and magnetic resonance imaging (MRI) has allowed us to visualize atherosclerotic plaque non-invasively. This has great appeal as they carry very little risk in comparison to invasive angiography and provide information of plaque composition in addition to stenosis severity.

The identification of plaques which are high-risk or ‘vulnerable’ to subsequent complications such as myocardial infarction or stroke would be highly valuable in our approach to incremental risk assessment and perhaps future treatment. Certain procedures in interventional cardiology such as saphenous vein graft (SVG) intervention and carotid stenting carry increased risk of embolic complications compared to coronary stenting. Non-invasive imaging could potentially identify certain plaque features which may be associated with an increased risk of embolization before embarking on such procedures. This thesis examines the utility of MDCT and MRI in atherosclerotic plaque imaging prior to SVG interventions and carotid stenting.

Our initial chapter investigates the angiographic parameters associated with embolization during SVG intervention. We correlate the amount of debris captured by distal protection devices during intervention with angiographic markers and subsequently, with impaired blood flow by way of Thrombolysis In Myocardial Infarction (TIMI) frame count.
Our next step involved the accuracy and reproducibility of MDCT and MRI in plaque quantification in comparison to our reference standard of intravascular ultrasound. We measured the luminal, vessel wall and plaque areas, and then calculated the resultant plaque volume of SVG lesions for all three modalities.

Having gained an understanding of the accuracy of MDCT and MRI, we investigated the relationships of MDCT plaque volume and density with embolic debris captured by distal protection device during SVG intervention. We then undertook histological assessment of the debris utilizing semi-automated image analysis software. We quantified the various plaque components including red blood cells, thrombus, lipid, cholesterol clefts and fibrous tissue. Finally, we explored the relationship between the histological findings with plaque volume, density and amount of embolization which occurred.

Our last original chapter investigates the utility of multi-weighted MRI to assess carotid plaque prior to stenting. We measured plaque volumes and characterized plaques as calcific, fibrotic or lipidic according to MRI findings. This information is then correlated to the amount of embolic debris captured by the distal protection device used during stenting.

In comparison to invasive imaging modalities like intravascular ultrasound, research into plaque characterization by MDCT and MRI is just beginning. Almost all of the current studies have been on coronary artery plaques. This thesis breaks new ground by studying SVG plaques and demonstrating links between plaque volume, composition and embolization during intervention. It builds on our knowledge of these non-invasive modalities and help us define their future roles.
DECLARATION

I declare that this thesis contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Gary Y. H. Liew. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University of Adelaide Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library catalogue, the Australasian Digital Thesis Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Gary Y. H. Liew
May 2012
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THESIS RELATED PUBLICATIONS


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THESIS RELATED PUBLISHED ABSTRACTS


6. **Liew GYH**, Hammett CJ, Dundon BK, Teo KSL, Worthley MI, Zaman AG, Worthley SG. Saphenous vein graft plaque quantification utilizing magnetic resonance imaging and multidetector computed tomography: A comparison with


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Travel Grant from National Heart Foundation of Australia to present at 57th Annual Scientific Sessions of the American College of Cardiology, Chicago, USA. 2008

Cardiac Society of Australia & New Zealand Travelling Fellowship to present at 58th Annual Scientific Sessions of the American College of Cardiology, Orlando, USA. 2009

Cardiovascular Lipid Travel Grant to present at 4th Annual Scientific Meeting of Society of Cardiovascular Computed Tomography, Orlando USA. 2010

Best Poster - Finalist, 55th ASM of Cardiac Society of Australia & New Zealand, Christchurch, New Zealand. 2007


Highly Commended Poster, University of Adelaide Research Expo, Adelaide, SA, Australia. 2008

Liew GYH, Hammett CJ, Dundon BK, Teo KSL, Worthley MI, Zaman AG, Worthley SG. Saphenous vein graft plaque quantification utilizing magnetic resonance imaging and multidetector computed tomography: A comparison with intravascular ultrasound
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACS</td>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>AMI</td>
<td>Acute myocardial infarction</td>
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<tr>
<td>CABG</td>
<td>Coronary artery bypass graft</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CAS</td>
<td>Carotid artery stenting</td>
</tr>
<tr>
<td>CDUS</td>
<td>Carotid Doppler ultrasound</td>
</tr>
<tr>
<td>CEA</td>
<td>Carotid endarterectomy</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross sectional area</td>
</tr>
<tr>
<td>CTA</td>
<td>Computed tomography angiography</td>
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<tr>
<td>DPD</td>
<td>Distal protection device</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiograph</td>
</tr>
<tr>
<td>EEM</td>
<td>External elastic membrane</td>
</tr>
<tr>
<td>EPD</td>
<td>Embolic protection device</td>
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<tr>
<td>FDG</td>
<td>Fluorodeoxyglucose</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>HU</td>
<td>Hounsfield Unit</td>
</tr>
<tr>
<td>IVUS</td>
<td>Intravascular ultrasound</td>
</tr>
<tr>
<td>LAD</td>
<td>Left anterior descending artery</td>
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<tr>
<td>LCx</td>
<td>Left circumflex artery</td>
</tr>
<tr>
<td>MACE</td>
<td>Major adverse cardiac events</td>
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<tr>
<td>MBG</td>
<td>Myocardial blush grade</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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<tr>
<td>MDCT</td>
<td>Multi-detector computed tomography</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>PCI</td>
<td>Percutaneous coronary intervention</td>
</tr>
<tr>
<td>PDW</td>
<td>Proton density weighted</td>
</tr>
<tr>
<td>QCA</td>
<td>Quantitative coronary analysis</td>
</tr>
<tr>
<td>RCA</td>
<td>Right coronary artery</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SVG</td>
<td>Saphenous vein graft</td>
</tr>
<tr>
<td>T</td>
<td>Tesla</td>
</tr>
<tr>
<td>TCFA</td>
<td>Thin cap fibroatheroma</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TFC</td>
<td>TIMI frame count</td>
</tr>
<tr>
<td>TIMI</td>
<td>Thrombolysis In Myocardial Infarction</td>
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<tr>
<td>TMPG</td>
<td>TIMI perfusion grade</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>T1W</td>
<td>T1 weighted</td>
</tr>
<tr>
<td>T2W</td>
<td>T2 weighted</td>
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Chapter 1

LITERATURE REVIEW
1.1 Introduction

Cardiovascular disease remains one of the leading causes of death in Australia and western societies. In Australia, it accounts for 35% of all deaths and affects 1.4 million Australians (6.9% of population) with a disability (AIHW 2008). Atherosclerosis is the predominant underlying cause of coronary artery disease (CAD), carotid artery stenosis and peripheral vascular disease (PVD). Atherosclerosis by itself is rarely fatal but it is the disruption of the plaque and superimposed thrombosis that leads to serious consequences such as acute myocardial infarction or stroke.

Whilst we have previously concentrated on the luminal stenosis of vessels as the main contributor of disease severity and perhaps subsequent clinical events, in recent times some have been paying more attention to the vessel wall and the plaque composition within it for features which may confer an increased risk of clinical sequelae.

The presence of the ‘vulnerable plaque’ consisting of a thin cap fibroatheroma with a large lipid pool has been put forward as the key ingredient for plaque rupture. This is particularly true for the coronary arteries. It forms one part of the bigger puzzle of the ‘vulnerable patient’ where multiple factors including inflammatory state, shear stress, cellular and local endothelial factors contributes to the risk of plaque rupture.

While we recognize that atherosclerosis is a systemic inflammatory problem, and some may look toward a systemic or even genetic solution, targeted local therapies for high risk plaques would have a role for some time into the future. We currently have a
range of invasive imaging tools that may identify these plaques but most are not widely available and carry a risk of complication due to their invasive nature. Therefore, the ultimate goal will be to identify these vulnerable plaques by non-invasive imaging techniques. We currently have little evidence to show targeted therapies to vulnerable plaques are efficacious, but we need to be able to first identify such a plaque should we choose to pursue this treatment paradigm.

It is clear from our experience of the last two decades of coronary and peripheral interventions that angioplasty and stenting can sometimes result in atheroembolic complications. There have been studies trying to identify predictors of such complications and subsequent technologies like distal protection devices to minimize the impact of atheroembolic material. Two areas which are particularly prone to such complications are saphenous vein graft and carotid artery stenosis interventions. The ability to non-invasively assess and identify plaques which are ‘high-risk’ for atheroembolism prior to interventions is an attractive concept. It may provide additional information for clinicians to tailor their interventional strategy or consider alternative therapies.
1.2 Atherosclerosis

1.2.1 Histology

Formation of atheroma begins with a fatty streak, an accumulation of lipid-laden inflammatory cells which later attract smooth muscle cells and extracellular matrix in the intima (Davies et al. 1988; Stary et al. 1994). Autopsy data show presence of some fatty streaks in aorta of all people aged 15 to 34 years old, its frequency increasing with age (McGill et al. 2000; Strong et al. 1999). Fatty streaks do not cause symptoms and may progress to atheroma or eventually disappear. Increased smooth muscle cell and lipid accumulation may lead to the formation of a fibrous plaque which has a predominance of connective tissue. As the atheroma progresses, it becomes revascularized and forms a necrotic lipid-rich core, some of which may eventually calcify. (Stary et al. 1995).

Table 1.2.1 outlines the American Heart Association (AHA) histological classification of plaque types (Stary et al. 1995). It includes an update containing Type VII and VIII which were previously listed under Type Vb and Vc (Stary 2000). It is thought that Type I-III are clinically silent and may regress to normal state or progress to more advanced types. The remaining plaque types may remain silent or be clinically overt but a plaque may undergo dynamic changes between Type V and VI as recurrent phases of thrombotic deposits and increased layers of fibrous tissue leads to worsening stenosis (Stary et al. 1995). A large number of vulnerable plaques are relatively bereft of calcium and not severely stenosed, similar to Type IV in the classification (Naghavi, Libby, et al. 2003).
Table 1.2.1  AHA classification of plaque types

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Main Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Initial lesion - isolated macrophage foam cells</td>
</tr>
<tr>
<td>Type II</td>
<td>Fatty streak - mainly intracellular lipid accumulation</td>
</tr>
<tr>
<td>Type III</td>
<td>Intermediate lesion – Type II changes &amp; small extracellular lipid pools</td>
</tr>
<tr>
<td>Type IV</td>
<td>Atheroma – Type II changes &amp; core of extracellular lipid</td>
</tr>
<tr>
<td>Type V</td>
<td>Fibroatheroma – lipid core &amp; fibrotic layer / multiple lipid cores</td>
</tr>
<tr>
<td>Type VI</td>
<td>Complicated – surface defect, haematoma / haemorrhage, thrombus</td>
</tr>
<tr>
<td>Type VII</td>
<td>Type V lesion - calcification predominates</td>
</tr>
<tr>
<td>Type VIII</td>
<td>Type V lesion - fibrous changes predominates</td>
</tr>
</tbody>
</table>

1.2.2 Pathogenesis

The pathogenesis of atherosclerotic plaque has been widely studied for many years with the predominant area being coronary artery disease. It is a multi-factorial process which includes inflammation, dyslipidaemia, endothelial dysfunction, infection and immunological factors (Hansson 2005; Mehta, JL et al. 1998; Ross 1999). While it is not the intention of this chapter to provide a comprehensive review of the various factors involved in the pathogenesis of atherosclerotic plaque, an overview may contribute to our understanding of the problem.
Figure 1.2.1 shows the postulate whereby inflammation and infection play important roles in the early initiation of endothelial injury. This leads to deposition of monocytes and lipid-laden macrophages into the subendothelial layers. Activation of inflammatory cells can lead to release of procoagulant cytokines and thrombosis. Increased release of free radicals and deficiency of antioxidant pool may oxidize lipids, inactivate nitric oxide and enhance thrombosis. Dyslipidaemia, altered folate metabolism and growth factor release cause smooth muscle cell proliferation and subsequent atherosclerosis (Mehta, JL et al. 1998).
1.2.2.1 **Flow & Mechanical Factors**

Despite the presence of known systemic risk factors (smoking, hypertension, diabetes, dyslipidaemia and family history), the propensity for formation of focal atherosclerotic plaques at branch points and bends suggests disturbances in blood flow patterns and low shear stress play a role. The vessel is subjected to mechanical forces and shear stress resulting from motion of the vessel and blood flow during cardiac cycle. These forces can stimulate the release of vasoactive substances, changes in cell metabolism and morphology (Hagiwara et al. 1998; Tsao et al. 1996). In the human carotid bifurcation where laminar blood flow is disrupted resulting in recirculating vortices, there is a strong correlation between endothelial dysfunction and low mean shear stress (Ku et al. 1985).

1.2.2.2 **Endothelial dysfunction**

Endothelial dysfunction appears to be an important initiating factor for subsequent events that ultimately lead to atherosclerosis. Possible causes include low mean shear stress, diabetes, smoking (free radicals), modified LDL, infections, genetic alterations or a combination of these and other factors (Ross 1999). The normal endothelium has a homeostatic role with a variety of endothelium-derived substances which may have atherogenic or atheroprotective effects. The dysfunctional endothelium will trigger a series of compensatory responses which result in increased cell adhesiveness, permeability and prothrombotic effects. If the initiating factor persists, the inflammatory response will continue, leading to migration and proliferation of smooth muscle and inflammatory cells. This cycle will eventually lead to growth of the atheroma.
A number of therapies have been shown to attenuate or reverse endothelial dysfunction: HMG-coenzyme A reductase inhibitor (statins) for hyperlipidaemia which increases bioavailability nitric oxide (John et al. 1998), ACE inhibitors (Mancini et al. 1996) and antioxidants eg. flavonoids in red wine (Stein, JH et al. 1999).

1.2.2.3  Inflammation

Inflammation is the central process in the development of atherosclerosis. It appears to involve both cellular and humoral pathways (Hansson 2005; Ross 1999). The ‘fatty streak’ which is a precursor to atheroma is an inflammatory lesion consisting of monocyte-derived macrophages and T lymphocytes (Stary et al. 1994). Macrophages in the dysfunctional endothelium release a number of cytokines, growth factors and inflammatory substance.

One of the first steps involved is leukocyte-endothelial adhesion which facilitates the entry and accumulation of inflammatory cells in vessel from their circulation in the blood stream. This is a multi-step process including leukocyte rolling, activation & arrest, and transmigration (Wagner & Frenette 2008). There are three families of adhesion molecules which are of particular importance:

Selectins are type 1 transmembrane glycoproteins located on both leukocytes and endothelial cells. They mediate cellular migration and slow rolling of leukocytes. They are named according to cell type which originally identified (E – endothelium, P – platelet and L – leukocyte).
Integrins are involved in leukocyte rolling but more importantly in arrest on the vascular endothelium. They are categorized according to the specific alpha and beta (1 & 2) chains present.

Immunoglobulin superfamily molecules are expressed on endothelial cells and interact with integrins on leukocytes for adhesion and transmigration. Of importance are intracellular adhesion molecule (ICAM-1, ICAM-2) and vascular cell adhesion molecule (VCAM-1). ICAM-1 is expressed at low levels and upregulated when the endothelium is stimulated by various factors eg. Interleukin-1 (IL-1), tumour necrosis factor-alpha (TNFα) and endotoxin. VCAM-1 is only synthesized after stimulation by IL-1, TNFα and endotoxin (Iiyama et al. 1999).

1.2.2.4 Dyslipidaemia

The association between lipid abnormalities or high serum cholesterol and increasing incidence of atherosclerosis has been demonstrated in large trials since the mid 1980s (Assmann & Schulte 1992; Stamler et al. 1986). High levels of low density lipoproteins (LDL) and low levels of high density lipoproteins (HDL) have been shown to be important risk factors (Gordon et al. 1977; NCEP 1988). There is an inverse relationship between level of HDL cholesterol and cardiovascular risk, with values above 1.9 mmol/L associated with better long-term outcome (Castelli 1983). Native LDL is thought not to contribute directly to the atherosclerotic process but it is oxidised LDL which is important in the initiation and progression of atherosclerosis (Witztum & Steinberg 1991). The specific mechanism of LDL modification is unclear but may include oxidation by nitric oxide synthase (NOS), myeloperoxidase and 15-lipoxygenase (Glass & Witztum 2001). Oxidised LDL is taken up by macrophages
(foam cells) in the atherosclerotic plaque via scavenger receptors (Iuliano et al. 2000). This may lead to mitochondrial dysfunction and necrosis with subsequent release of inflammatory cytokines and prothrombotic factors (Tabas 2002). Thus, leading to the process of endothelial dysfunction and inflammation discussed earlier. Oxidized LDL itself may cause disruption of endothelial cells and have a role in plaque instability with increased levels found in patients with acute coronary syndrome (Ehara et al. 2001).

Hypertriglyceridemia as a contributor to atherosclerosis have been proposed in a number of epidemiologic and clinical studies over the years but remain difficult to prove. It has an association with low HDL and many triglyceride lowering therapies have beneficial effects on other lipid parameters (Le & Walter 2007). The risk of cardiovascular disease appear to increase when triglyceride levels are above 2.3 mmol/L.

1.2.2.5 Infection

The relationship between infection and atherosclerosis is a controversial one. Apart from early anecdotal evidence, the first serious link was established in the late 1970s where avian herpes virus in smooth muscle cells caused cholesterol accumulation and atherosclerotic-like lesions in chickens (Fabricant et al. 1983; Minick et al. 1979). Subsequently, human studies found pathogens in atherosclerotic vessels (Chiu et al. 1997; Kuo et al. 1993) and associations were established between pathogen-specific antibodies and atherosclerosis (Saikku et al. 1988; Thom et al. 1992). The main organisms implicated were: Chlamydia pneumonia, cytomegalovirus (CMV), herpes
simplex virus (HSV), coxsackie B virus, hepatitis A virus (HAV) and *Helicobacter pylori*.

The mechanisms whereby infection can contribute to atherosclerosis include direct effects on vessel, persistent abortive infections and induction of a systemic inflammatory state. CMV, HSV and *Chlamydia pneumonia* can reside in cells of vessel wall causing endothelial dysfunction and subsequent increased production of cytokines, adhesion molecules and oxidised LDLs (Epstein et al. 1999). Persistent cellular infection with *Chlamydia pneumonia*, although in a quiescent state, can also produce a heat shock protein – HSP60, which have pro-atherogenic activity (Kol et al. 1999).

Although there have been numerous studies since showing an association between various pathogens and atherosclerosis, they have been largely observational rather than proving causality in human atherosclerosis. In addition, there were other sero-epidemiological studies which did not find any link between infection and atherosclerosis (Ridker et al. 1999; Zhu et al. 2002). Only in animal studies, especially those utilizing apolipoprotein E (apo E) knockout mice which showed that infection can cause injury to endothelial cells and induce atherosclerosis (Naghavi, Wyde, et al. 2003; Span et al. 1989). Furthermore, chronic infection with CMV or *Chlamydia pneumonia* in apoE knockout mice can increase atherosclerotic lesion size (Ezzahiri et al. 2002; Hsich et al. 2001). It is still uncertain if such results apply to humans and demonstrating the same process prospectively would be problematic.
If infections do indeed have a link to atherosclerosis, one may expect the pathogen load or burden to correlate with diseases associated with atherosclerosis. Studies have shown correlations between the number of infecting pathogens with increasing CAD risk, C-reactive protein (CRP) levels and endothelial dysfunction (Prasad et al. 2002; Zhu et al. 2000). In two prospective studies of patients with CAD, the pathogen burden predicted incidence of AMI and death (Rupprecht et al. 2001; Zhu et al. 2001).

The concept of treating infections with antimicrobials in the effort to reduce atherosclerotic disease has also been tested. These studies concentrated on using macrolide antibiotics against *Chlamydia pneumonia* as it seemed to be the most important pathogen involved. Unfortunately, meta-analyses of these prospective, randomized controlled trials did not show any effect on clinical outcomes such as AMI or death (Andraws et al. 2005; Etminan et al. 2004). Due to the disappointment of these antibiotic trials at the time, the idea of infections contributing to atherosclerosis development seemed to have lost momentum.

However, there are arguments that the endpoints of those studies concentrating on acute events may be flawed as applied to *Chlamydia pneumonia*. Predictive information for AMI / death by pathogen burden was largely related to viruses (Rupprecht et al. 2001). Bacterial burden correlated with anatomic severity of CAD but not acute events (Espinola-Klein et al. 2002). Moreover, studies seem to implicate that patients are infected with multiple pathogens which could play different roles in the process of atherosclerosis (Rupprecht et al. 2001; Zhu et al. 2000). Therefore, the failure to show an impact on acute outcomes in the treatment of one organism may not be sufficient to dismiss the role of infections in atherosclerosis.
1.2.3 Arterial Remodelling

The continuing growth of atheroma within the intima of the vessel was thought to always lead to gradual luminal narrowing. However, Glagov et al. demonstrated the concept of ‘positive remodelling’ in an autopsy study that certain plaques may lead to an expansion of the media and external elastic membrane, preserving the lumen size (Glagov et al. 1987). This expansion of the artery could occur until the lesion reached 40% area stenosis, above which the lumen starts to narrow (Figure 1.2.2).

Figure 1.2.2. Early plaque accumulation in human coronary arteries is associated with compensatory enlargement of vessel size (positive remodelling). Therefore, luminal size is initially not affected by plaque growth. The complex changes of lumen, plaque and external elastic membrane (EEM) may also affect plaque regression. Schoenhagen, P, J Am Coll Cardiol, 2001; 38: 297-306.
Since the landmark paper by Glagov, there have been in vivo studies of human coronaries using intravascular ultrasound (IVUS) with similar findings. The external elastic membrane (EEM) area in segments of ‘positive remodelling’ were larger than the adjacent reference site (Ge et al. 1993). In some cases, the remodelling can be negative with the EEM being smaller than the reference site (Figure 1.2.3). Positive remodelling has been defined as when the EEM of the lesion site is larger than proximal reference site with a remodelling ratio (RR) of greater than 1.05. Conversely, negative remodelling is when the RR is less than 0.95 (Schoenhagen et al. 2001).

![Figure 1.2.3](image)

**Figure 1.2.3.** Top panel shows ‘positive remodelling’ where the EEM of the lesion has a RR >1.05 compared to the proximal reference site. The bottom panel shows ‘negative remodelling’ where the EEM of the lesion has a RR<0.95. Schoenhagen, P, J Am Coll Cardiol, 2001; 38: 297-306.

Although positive remodelling was initially viewed as a compensatory mechanism in early atherosclerosis, subsequent studies have explored its association with unstable clinical presentation. Utilizing IVUS and angioscopy, studies have found unstable or complex
coronary lesions to have positive remodelling rather than negative remodelling (Schoenhagen et al. 2000; Smits et al. 1999). The converse was also true of stable lesions. The pathophysiologic mechanisms are still unclear but one hypothesis is that positive remodelling is a characteristic of plaque inflammation and proliferation (Yamagishi et al. 2000). These plaques may be prone to rupture leading to acute coronary syndromes as opposed to lesions with negative remodelling that are largely fibrotic. A small IVUS study involving three years of follow-up and pravastatin treatment showed an increase of plaque area by 41% in the control group but a decrease of 7% in the treatment group (Takagi et al. 1997). The EEM area increased in the control group but not the treatment group. This suggests that plaque regression may involve changes in plaque size and perhaps composition, leading to negative remodelling.

1.2.3.1 **Saphenous vein graft remodelling**

The IVUS studies examining remodelling in coronary bypass grafts have shown mixed results. One study found significantly smaller lumen size at lesion sites but did not find positive remodelling (Nishioka et al. 1996). However other studies have found the EEM area was larger in up to 96% of lesions with a significant correlation between plaque and EEM area (Mendelsohn et al. 1995) or even both positive and negative remodelling may occur (Hong, Mintz, et al. 1999). The varied results may be due to the age of saphenous vein grafts (SVG) studied in different patients rather than serial observations. Lau et al, showed in serial measurements utilizing computed tomography (CT) and IVUS that the lumen loss of SVGs in their first year is a result of negative remodelling rather than changes in wall thickness (Lau et al. 2006). However, the studies mentioned earlier
involved patients with grafts which were up to 20 years old and there is little serial data on remodelling in such old grafts.

### 1.2.4 Vulnerable Plaque

Plaque rupture and thrombosis is thought to be the predominant mechanism which leads to clinical events such as myocardial infarction. The progression from normal coronary arteries to vulnerable plaques and clinical events may adopt the following pathways (Figure 1.2.4)

![Diagram of atherosclerosis progression](image)

**Figure 1.2.4.** Development of atherosclerosis and progression to thrombosis and clinical events. The later stages of progression is unpredictable and may be recurrent. Adapted from Schaar, JA. Eur Heart J. 2004; 25:1077-1082.
The usage of the term “vulnerable plaque” has been first attributed to Muller and colleagues in their investigation of triggers of coronary thrombosis and circadian variation (Muller et al. 1989). Since then it has been recommended to identify all thrombosis-prone plaques and plaques with a high probability of undergoing rapid progression to culprit plaques (Naghavi, Libby, et al. 2003). A meeting convened in June 2003 in Santorini, Greece attended by eminent investigators on vulnerable plaque proposed a set of terminology (Table 1.2.2) for lesions associated with the progression of atherosclerosis (Schaar, Muller, et al. 2004).
### Table 1.2.2 Definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culprit lesion</td>
<td>A lesion in a coronary artery considered, on the basis of angiographic, autopsy or other findings, to be responsible for the clinical event. In unstable angina, myocardial infarction and sudden coronary death, the culprit lesion is often a plaque complicated by thrombosis extending into the lumen.</td>
</tr>
<tr>
<td>Eroded plaque</td>
<td>A plaque with loss and/or dysfunction of the luminal endothelial cells leading to thrombosis. There is usually no additional defect or gap in the plaque, which is often rich in smooth muscle cells and proteoglycans.</td>
</tr>
<tr>
<td>Vulnerable / High-risk / thrombosis prone plaque</td>
<td>These terms can be used as synonyms to describe a plaque that is at increased risk of thrombosis (or re-thrombosis) and rapid stenosis progression.</td>
</tr>
<tr>
<td>Inflamed thin-cap fibroatheroma (TCFA)</td>
<td>An inflamed plaque with a thin cap covering a lipid-rich, necrotic core. An inflamed TCFA is suspected to be a high-risk/vulnerable plaque.</td>
</tr>
<tr>
<td>Plaque with calcified nodule</td>
<td>A heavily calcified plaque with the loss and/or dysfunction of endothelial cells over a calcified nodule, resulting in loss of fibrous cap, that make the plaque at high-risk/vulnerable. This is the least common of the three types of suspected high-risk/vulnerable plaques.</td>
</tr>
<tr>
<td>Ruptured plaque</td>
<td>A plaque with deep injury with a real defect or gap in the fibrous cap that had separated its lipid-rich atheromatous core from the flowing blood, thereby exposing the thrombogenic core of the plaque. This is the most common cause of thrombosis.</td>
</tr>
<tr>
<td>Thrombosed plaque</td>
<td>A plaque with an overlying thrombus extending into the lumen of the vessel. The thrombus may be occlusive or non-occlusive.</td>
</tr>
<tr>
<td>Vulnerable patient</td>
<td>A patient at high-risk (vulnerable, prone) to experience a cardiovascular ischemic event due to a high atherosclerotic burden, high-risk/vulnerable plaques, and/or thrombogenic blood.</td>
</tr>
</tbody>
</table>

It has been proposed that the most susceptible and common histological type of ‘vulnerable plaque’ is the thin-capped fibroatheroma (TCFA) (Kolodgie et al. 2001). Figure 1.2.5 demonstrates some of the features of a TCFA: (i) the thickness of the fibrous cap is < 65 µm, (ii) large lipid-rich necrotic core and (iii) activated macrophages within the fibrous cap (Burke, AP et al. 1997).
Figure 1.2.5. Example of a thin-cap fibroatheroma in a coronary artery; it is eccentric and presumed to be rupture prone. Components are highlighted as: large lipid-rich necrotic core (orange asterisk), thin fibrous cap (blue arrows), expansive / positive remodelling (green arrow) and vasa vasorum with neovascularisation (red open circles). (Falk 2006)

From a recent pathologic study of sudden cardiac death, we can appreciate that TCFAs have morphological characteristics that most closely resemble ruptured plaques (Virmani et al. 2000). The necrotic core occupies a large area of the plaque which is rich in cholesterol clefts or lipid, and the increased macrophage which suggests the active role of inflammation in plaque rupture (Table 1.2.3).
Table 1.2.3  Morphological characteristics of culprit and rupture-prone plaques in sudden cardiac death

<table>
<thead>
<tr>
<th>Plaque Type</th>
<th>Necrotic Core (%)</th>
<th>Cholesterol Clefts (%)</th>
<th>Macrophages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rupture</td>
<td>34±17</td>
<td>12±12</td>
<td>26±20</td>
</tr>
<tr>
<td>TCFA</td>
<td>23±17</td>
<td>8±9</td>
<td>14±10</td>
</tr>
<tr>
<td>Erosion</td>
<td>14±14</td>
<td>2±5</td>
<td>10±12</td>
</tr>
<tr>
<td>Fibrocalcific</td>
<td>15±20</td>
<td>4±6</td>
<td>6±8</td>
</tr>
</tbody>
</table>

Data adapted from Virmani, R, Arterioscler Thromb Vasc Biol 2000; 20:1262-1275

It is important to note that ‘vulnerable plaque’ represents a functional definition rather than a fixed histological entity. There are other histological types of vulnerable plaque which include eroded plaque, intra-plaque haemorrhage, plaque with calcified nodule and critically stenotic plaques (Naghavi, Libby, et al. 2003).

The eroded plaque was first described in an autopsy series of sudden cardiac death with prevalence as high as 44% (Farb et al. 1996). There is thrombus overlying the lesion but no structural defect or gap in the plaque which is rich in proteoglycans and smooth muscle. The plaque luminal surface is irregular, eroded and lacking in endothelial cells; there is often very little or no lipid pool present. In comparison with plaque rupture, erosions are more common in women and younger patients. The lesions are less stenotic (70% v 78%), calcification less common (23% v 69%) and infiltration by macrophages and T cells are half of plaques which rupture (Farb et al. 1996)
1.2.4.1 Plaque Rupture

Plaque rupture is the most common complication of atherosclerosis which results in fatal AMI or sudden coronary deaths (Davies & Thomas 1984; Virmani et al. 2000). It accounts for about 60-70% of the culprit lesions with the remaining of un-ruptured plaques consisting of plaque erosions (30%), calcified nodules (2-7%) and others (Naghavi, Libby, et al. 2003).

Factors found to be involved in the atherosclerotic plaque ruptures include:

(i) Size and consistency of the atheromatous core
(ii) Thickness, collagen and smooth muscle content of the fibrous cap
(iii) Inflammation within the cap
(iv) Cap fatigue – repetitive stress by flexion, shear and pressure during cardiac cycle

Autopsy studies into AMI demonstrated ruptured plaques containing larger necrotic cores (Gertz et al. 1991; Kragel et al. 1989) while a study on aortic plaque showed a necrotic core area threshold of >40% being at high risk of rupture (Davies et al. 1993).

The site of plaque rupture is often at the shoulder of the thin fibrous cap (Figure 1.2.6). This was particularly evident in eccentric plaques where 63% of the ruptures occurred at the junction of the plaque cap with normal intima (Richardson et al. 1989). The same study used computer modelling to demonstrate maximal stress was found at that point.
Figure 1.2.6. Cross section of coronary artery showing ruptured plaque. The lumen (asterisks) is occupied by thrombus (Th) formed when the fibrous cap (FC) has ruptured at the shoulder (arrow), allowing the contents of the lipid core (LC) which is highly thrombogenic, into the lumen. Adapted from Constantinides P. Am J Cardiol. 1990; 66:37G.

Subsequent studies have found that inflammation may have a key role in plaque rupture. An active inflammatory process involving macrophages was always found at sites of rupture in patients who died from AMI, irrespective of the plaque morphology involved (van der Wal et al. 1994). Activated macrophages were shown to induce collagen breakdown in fibrous caps by way of matrix metalloproteinases (MMPs) secretion (Shah, PK et al. 1995). Similarly, activated mast cells which contain proteases that can trigger degradation of the extracellular matrix via activation of MMPs were found in concentrations 200-fold more than normal in sites of erosion or rupture in patients who died from AMI (Kovanen et al. 1995).
**1.2.4.2  Plaque size and composition**

A series of prominent angiographic studies done in patients prior to AMI found that a large proportion of culprit lesions had angiographic diameter stenosis of <50% (Ambrose et al. 1988; Giroud et al. 1992; Little et al. 1988). It was suggested that up to 68% of AMI occurred in lesions of <50% stenosis and only 14% occurred with lesions of >70% severity (Figure 1.2.7) (Falk et al. 1995). However, subsequent angiographic studies such as the Coronary Artery Surgery Study (CASS) have demonstrated that the rate of vessel occlusion or cardiac events rises exponentially with the baseline stenotic severity (Alderman et al. 1993). One explanation given at the time was while occlusion increases with stenosis severity, mild to moderate lesions were far more common than severe ones and therefore contribute to an overall larger number of AMIs or cardiac events (Falk et al. 1995).

![Figure 1.2.7](Image)

**Figure 1.2.7.** Bar graphs showing the angiographic trials of culprit lesions and the varying degree of stenosis prior to AMI. Adapted from Falk et al. Circulation. 1995; 92:657-71.
This gave rise to the notion that less obstructive lesions were more prone to rupture, precipitating AMI or death and may represent vulnerable plaques with lipid-rich core compared with more stenotic ones (Little et al. 1988; Nobuyoshi et al. 1991). A less stenotic lesion is also more likely to lead to clinical events after abrupt occlusion compared to severely stenotic ones due to lack of collateral channels development (Epstein 1988; Juilliere et al. 1990).

However, this is discordant with the majority of autopsy data which has traditionally demonstrated that plaque rupture and thrombosis occurred in lesions with severe stenosis (Davies & Thomas 1984; Falk 1983; Qiao & Fishbein 1991). These studies used planimetry to determine the area which plaque occupies the cross sectional area (CSA) – typically this was 90% (Figure 1.2.8).
How do we reconcile the different observations? Firstly, the two techniques are not measuring the same thing – angiography gives us diameter stenosis while histology / planimetry give us an area stenosis. A lesion causing the luminal diameter to halve will result in a 50% diameter stenosis but a 75% CSA stenosis by simple arithmetic. The implications will be discussed further in the context of remodelling. Secondly, the intervals between angiogram and AMI in studies listed in Figure 1.2.7 were up to 2 years, allowing the possibility of plaque growth and stenosis progression. Indeed, a more recent study found a mean diameter stenosis of 71% in culprit lesions 3 days prior to AMI (Ojio et al. 2000).
While there may be a multitude of factors which contribute to inaccurate angiographic or histological measurements, one needs to consider two important processes:

- vascular remodelling
- diffuse nature of coronary atherosclerosis.

As discussed earlier, positive remodelling may result in the cross-sectional area increase by 40% before a change in lumen size is seen. Coronary atherosclerosis is often diffuse; it may be underestimated by angiography and indeed look normal on angiograms (Leung et al. 1995). The diffuse nature of atherosclerosis has been shown in studies of pathology (Arnett et al. 1979; Thomas, AC et al. 1986; Warnes & Roberts 1984) and IVUS (Mintz et al. 1995; Porter et al. 1993).

The diameter stenosis determined by angiography depends on the comparison with a reference segment which is thought to be normal. However this reference segment may often be diseased and therefore lead to underestimation of the stenosis severity. Conversely in histology assessments, one is comparing the lumen with total plaque area, positive remodelling may lead to an overestimation of the degree of luminal stenosis (Fishbein & Siegel 1996). Furthermore, there is no comparison with a reference segment of normal histology.

It is important to remember that angiography is good at demonstrating the lumen but not the plaque volume or burden. It does not show the outer vessel wall or external elastic membrane in order to demonstrate vascular remodelling. A lesion may contain a large area of plaque with large necrotic core and yet only have mild encroachment of the lumen. A study using IVUS demonstrated that plaque rupture seldom occurs at sites with minimal disease but at sites of significant plaque accumulation and
remodelling (Fujii et al. 2006). The study found that ruptured plaques contain large lipid pool, plaque burden and the degree of lumen compromise to be variable and often insignificant.

In a more recent autopsy study of 38 hearts, approximately 75% of arteries with TCFA and ruptured plaques had CSA stenosis of less than 75% (Virmani et al. 2002). The mean CSA stenosis for TCFA was 60% and for ruptured plaques was 73%. The same group had previously found that in sudden cardiac death, there was only a moderate degree of stenosis (74% CSA) in thrombosed arteries (Farb et al. 1996). The authors noted that only a third of their patients had histological evidence of AMI, suggesting heterogeneity in lesion severity contributing to sudden cardiac death and AMI. Their data would support the concept that lesions <50% diameter stenosis may be vulnerable and lead to adverse outcomes.

There is a concept that atherosclerosis is a pan-coronary process. Pathology and angiographic studies have shown that when one ruptured plaque is present, another is found in 30-40% of cases (Cheruvu et al. 2007; Goldstein et al. 2000). However, not every rupture results in a clinical event. Ruptures can be silent and repetitive, interspersed with healing and thrombus formation (Burke, AP et al. 2001; Mann & Davies 1999). Indeed, the degree of luminal narrowing increases with each successive healed rupture. A small study using IVUS has shown good results with medical therapy of these non-culprit 2\textsuperscript{nd} rupture plaques with a 15% reduction in stenosis at 2 years (Rioufol et al. 2004).
The majority of plaque ruptures occur in the proximal to mid large coronary vessels (el Fawal et al. 1987). A recent pathologic study of cardiovascular deaths found that 90% of ruptured plaques and TCFA of the left coronary system occurred within the first 30mm (Cheruvu et al. 2007). The location of acute coronary occlusions during STEMIs on angiograms were found to cluster within the proximal third of each major coronary vessel (Wang, JC et al. 2004). Furthermore, the calculated risk of infarct decreases by each 10-mm segment away from the ostium. Similarly, studies using IVUS found that most plaque ruptures occurred in the proximal to middle segments of coronary vessels (Fujii et al. 2006; Hong et al. 2005). This was been confirmed with newer invasive imaging techniques of ‘virtual histology’ (Hong et al. 2008) and optical coherence tomography (Tanaka, Imanishi, et al. 2008). The proximal nature of the lesions means that we have the option of focal treatment via PCI should that be appropriate.
1.2.4.3 CONCLUSIONS

Atherosclerosis is an inflammatory disease of multiple causes. The concern with atherosclerosis is the development of sudden adverse events when a high-risk or ‘vulnerable’ plaque ruptures and results in thrombosis. It can occur in positively remodelled lesions which are not tightly stenosed. From autopsy studies, we know occlusive and sometimes non-occlusive thrombosis can lead to sudden cardiac death and AMIs. The TCFA has been postulated to be the precursor of plaque rupture and contain certain morphological features which could be targets for identification via either invasive or non-invasive imaging. Furthermore, plaque rupture does not always result in adverse events but can be silent and undergo multiple cycles of healing and rupture, providing further opportunity for detection and perhaps treatment. Invasive angiography is not particularly good at detecting TCFA or vulnerable plaques as it primarily assesses luminal stenosis. Therefore, we must look toward other imaging methods of identifying the vulnerable plaque.
Invasive angiograms provide excellent information on the lumen of a vessel but as discussed previously, have limitations in providing information on plaque composition. Angiographic disease progression is a weak predictor of AMI or death (Waters et al. 1993). There is clearly a detection gap in coronary artery disease prognosis and the size of this is unknown but likely to be substantial (Pasternak et al. 2003). The current tools we use for this and how they are applied are imperfect. Therefore, the opportunities exist for improvement in the area of imaging. The identification of high-risk plaques will likely require an invasive approach in select individuals in the foreseeable future but a practical solution for the longer term would be via non-invasive imaging (Braunwald 2006).

The identification of vulnerable plaques may come from two broad areas – anatomical and functional. Morphological features of TCFA such as remodelling, large necrotic core and thin fibrous cap are already demonstrable with certain invasive and non-invasive imaging techniques. The central role which inflammation plays in the process leading to plaque instability and rupture provides the basis for detecting inflammatory activity or changes in temperature. We will explore briefly the role of each technique and expand on those which are more relevant to this thesis (Table 1.3.1)
<table>
<thead>
<tr>
<th>Invasive</th>
<th>Non-Invasive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravascular ultrasound (IVUS)</td>
<td>Multidetector computed tomography (MDCT)</td>
</tr>
<tr>
<td>Virtual Histology (VH)</td>
<td>Magnetic resonance imaging (MRI)</td>
</tr>
<tr>
<td>Optical coherence tomography (OCT)</td>
<td>Positron emission tomography (PET)</td>
</tr>
<tr>
<td>Angioscopy</td>
<td></td>
</tr>
<tr>
<td>Intravascular Palpography</td>
<td></td>
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<tr>
<td>Thermography</td>
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<tr>
<td>Near IR spectroscopy (NIRS)</td>
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### 1.3.1 Invasive Techniques

#### 1.3.1.1 Intravascular Ultrasound

Intravascular ultrasound (IVUS) is an invasive catheter based technique which provides high resolution cross sectional images of a vessel. It is capable of providing highly accurate measurements of vessel lumen, plaque and vessel area delineated by the external elastic membrane (Mintz et al. 2001). Therefore, it is excellent at quantifying plaque burden or volume and documenting vascular remodelling.

Pathologic studies have suggested that the extent of atherosclerosis has been underestimated in ‘angiographically normal’ segments of coronary vessels. An important IVUS study conducted by Mintz et al, showed only 6.8% of these angiographically normal reference segments were free from disease on IVUS (Mintz et al. 1995). These ‘normal’
segments actually had an average cross-sectional area narrowing of 51% on IVUS and was largely due to plaque which contained little calcium or fibrous tissue.

Consequently, guidelines on the performance and interpretation of IVUS images has been published (Mintz et al. 2001). The proximal reference is the largest lumen proximal to a stenosis within the same segment (usually within 10mm) but this may not correspond to the site with the least plaque. Similarly, the distal reference point is the largest lumen distal to the stenosis within the same segment, and often used when dealing with ostial lesions. There are a number of artefacts which may impact on the image analysis including motion, non-uniform rotational distortion, ring-down, blood speckle, etc. One particular problem occurs with larger vessels where the transducer may not be sitting in the middle of the vessel resulting in an oblique image which over-estimates the dimensions.

The ability of IVUS to characterize plaque has been studied extensively. Given the grey-scale nature of ultrasound, its ability to detect and quantify specific histologic contents is limited as some components may have similar echogenicity. It is excellent at detecting calcium but cannot determine its thickness as ultrasound cannot penetrate calcium (Tuzcu et al. 1996). This often results in a bright arc and corresponding acoustic shadow behind it which we can’t derive any information. Conversely, tissue or plaque which contains mostly lipid will be very echolucent or dark (Nishimura et al. 1990). Fibrous plaques then to have a common type of lesion found. However, an echolucent zone within a plaque may represent necrotic core, intramural haemorrhage or thrombus. The diagnosis of thrombus can be difficult as the features may be variable. It is usually an intraluminal mass with a layered, lobulated or pedunculated appearance (Siegel et al. 1991).
In acute coronary syndrome (ACS), IVUS has been used to demonstrate vulnerable plaques. It was found that culprit plaques have greater plaque burden, eccentricity, echolucency, thrombus and positive remodelling (Fujii et al. 2003; Maehara et al. 2002). Plaque ruptures may happen in non-culprit lesions and in multiple locations. Hong et al. demonstrated plaque rupture at culprit lesions in 66% of AMI patients and 27% of stable angina patients. Ruptures were also found in lesions of non-infarct related arteries of 17% of AMI patients and 5% of stable angina patients (Hong et al. 2004). Other similar studies found a quarter of patients had ruptures in more than one coronary artery (Tanaka et al. 2005), and up to 79% of patients may have ruptured plaques in non-culprit arteries (Rioufol et al. 2002).

While the characteristics described by IVUS seems to fit for TCFA and hence the identification of vulnerable plaques, they have not been definitive. The vast majority of studies have been retrospective and they differ on the characteristics of culprit lesions. A rare prospective study by Japanese researchers identified plaque burden, echoluent zones and eccentricity as predictors of subsequent rupture and ACS (Yamagishi et al. 2000). However, a study also found culprit lesions of ACS were very similar to those with only stable angina (Schoenhagen et al. 2003). Therefore, the ability to identify plaques which will eventually become culprit plaques is still somewhat elusive.

Due to the accuracy of IVUS in measuring plaque volume, it has been used in a number of clinical trials in assessing plaque progression as a primary endpoint. While IVUS provides up to 80 frames per mm of vessel, convention has been to analyse images 1 mm apart and summate the areas to the total length of the lesion, thus providing a volume of the plaque (Nicholls et al. 2010). High dose statins have been shown to slow disease progression.
Nissen et al. 2004) and even plaque regression (Nissen et al. 2006) as measured by IVUS. In a pooled analysis of 6 serial IVUS trials involving over 4,000 patients, the burden of coronary artery disease at baseline and its rate of progression were found to be independently associated with adverse outcomes (Nicholls et al. 2010).

With regards to IVUS of SVG, there are some morphological differences. Veins do not have an EEM but SVG typically undergo arterialization which involves intimal fibrous thickening, medial hypertrophy and lipid deposition. There is a sonolucent zone which develops and the outer border is traced which is analogous to the EEM area (Mintz et al. 2001).

1.3.1.2 Virtual Histology

Virtual Histology (VH) is based on grayscale IVUS but uses spectral analysis of ultrasound backscatter to provide information of plaque composition. This has been validated with histology on coronary arteries at autopsy in classifying lesions as lipid-rich, fibro-fatty, calcified with necrotic core or calcified (Nair et al. 2002). Similarly, VH has been validated in carotid artery plaques prior to endarterectomy (Diethrich et al. 2007).

The extra information gain on plaque composition may aid in future endeavours to identify vulnerable plaques. In a small in-vivo study, VH was able to demonstrate the size of necrotic core to be significantly larger in coronaries with positive remodelling (Rodriguez-Granillo et al. 2006). In another study of acute coronary syndromes, investigators
demonstrated correlation between fibro-fatty plaque volume and slow flow during PCI (Bae et al. 2008).

In the largest prospective study to date, the PROSPECT trial studied 697 patients with acute coronary syndrome with VH and followed for median of 3.4 years to determine subsequent major adverse cardiovascular events (Stone, Maehara, et al. 2011). They found the majority of subsequent events were attributable to plaques which had thin cap and large atheroma burden even though they were angiographically mild at the time.

### 1.3.1.3 Optical Coherence Tomography

Optical coherence tomography (OCT) is a catheter based imaging technique that provides high resolution cross-sectional images of the vessel wall via measurements of back-reflection of infrared light. Compared to IVUS, it provides a 10-fold increase in resolution (10-20 µm) utilizing finer catheters, typically 0.017-inch, as they do not require a transducer within the catheter (Jang et al. 2002). The main drawbacks of OCT are the limited penetration in tissue (2-3 mm) and attenuation by blood, often requiring proximal balloon occlusion or saline / dextran flushes. Recently, the next generation of frequency domain OCT has become available with much greater frame rate and pullback speed, enabling the use of conventional contrast agent as a flush without vessel occlusion (Takarada et al. 2010).

In the 1990s, OCT has undergone comparison studies with IVUS and histology (Brezinski et al. 1997; Fujimoto et al. 1999; Patwari et al. 2000). OCT was qualitatively superior to
IVUS in demonstrating structural detail due to its higher resolution. The markers for OCT to identify TCFA / vulnerable plaque currently are: cap thickness, plaque characteristics and macrophage content which is represented by granularity.

The ability of OCT to measure intimal cap thickness has been a strong point of the technique which was validated with histology (Jang et al. 2005; Yabushita et al. 2002). The high resolution images allow it to excel in detection of plaque rupture in vivo when compare with other techniques like IVUS and angioscopy (Kubo et al. 2007). Plaque characterization of human coronaries report sensitivity and specificity above 90% with good reproducibility when compared to histology (Yabushita et al. 2002). A recent study comparing OCT to IVUS found OCT to be superior in distinguishing calcific, fibrous and lipidic plaque (Kawasaki et al. 2006). However, one has to keep in mind that this is only the shallow portion of plaque which is visible to OCT. It also has the ability to quantify macrophage density in plaque fibrous caps with a high degree of correlation with CD68 immunoperoxidase staining on histology (Tearney et al. 2003). The macrophage density was greater in TCFA and also correlated with peripheral white blood cell count (Raffel et al. 2007)

OCT can also be used in the setting of PCI to assess adequate stent apposition and neointima coverage of stent during follow-up to minimize the risk of stent thrombosis post drug eluting stent implantation (Suzuki et al. 2008).
1.3.1.4 **Angioscopy**

Angioscopy is the only *in vivo* technique that allows direct visualization of the luminal surface of the vessel via a fibre-optic bundle in the catheter. It can provide detailed characterization of plaque surface colour, integrity and presence of thrombus. The technique requires displacement of blood with saline flushing and often proximal balloon occlusion which has the potential of ischaemia and vascular injury. It often requires a larger guiding catheter (7 French) and can only study relatively large coronary arteries. Inspection of the lesion may be hindered by distal location or inability to cross tight lesions.

The colour of plaque seen with angioscopy has been correlated with histology. They can range from white (fibrous) to yellow (lipidic) and gray-yellow (degenerated plaque) (den Heijer et al. 1994; Thieme et al. 1996). The degree of yellow saturation in a plaque may reflect its location towards the surface, representing a thin fibrous cap rather than the amount of lipid present (Kubo et al. 2008). The ‘glistening yellow’ colour was found to be a predictor of future myocardial infarction (Uchida et al. 1995) and it is the usual colour of the plaque underlying intracoronary thrombus (Waxman et al. 1997). Therefore, there is the potential of angioscopy to detect vulnerable plaques, or at least TCFAs.

The ability for angioscopy to detect intracoronary thrombus is a strong point. Studies found thrombus in over 80% of culprit lesions of AMI, 50% of lesion in unstable angina and only 15% of stable angina (de Feyter et al. 1995; Sherman et al. 1986; Van Belle et al. 1998). Plaque disruption can also be detected with a positive predictive value of over 90% when compared with histology (Siegel et al. 1991). Angioscopic studies often describe additional
yellow plaques in acute coronary syndrome but very low incidence of a second plaque
disruption. A second ulcerated plaque was found in 10% (Uchida et al. 1995) and a second
thrombus in only 2% (Asakura et al. 2001).

1.3.1.5 Intravascular Palpography

Palpography is a research tool based on IVUS which assess the deformation of tissue or
plaque caused by change in intraluminal pressure using a compliant intravascular balloon
(Schaar et al. 2006). The premise is based on the difference in mechanical properties
between fibrous and lipidic plaque and the hypothesis of a TCFA being prone to rupture
due to increase strain on the cap (Loree et al. 1992). An early ex-vivo study on coronaries
and femoral arteries found highly significant differences in strain of fibrous, fibrous/fatty
and fatty plaques (de Korte et al. 2000).

Detection of vulnerable plaque has been tested in ex-vivo coronary arteries (Schaar et al.
2003). When compared to histology, palpography had a sensitivity of 88% and specificity
of 89%. They found high strain areas to correspond to the shoulder regions of eccentric
plaques along with macrophage infiltration. In vivo validation has been performed in pigs
(de Korte, Sierevogel, et al. 2002), and early studies in humans showed high strain in
plaques of patients with acute coronary syndrome (de Korte, Carlier, et al. 2002; Schaar,
1.3.1.6  Thermography

The hypothesis behind this invasive technique is that plaques with active inflammation are hotter than normal arteries or quiescent plaques. The presumption is that these hot plaques are vulnerable plaques which may lead to clinical events. There are a number of different catheter based devices ranging from simple single temperature sensor (Stefanadis et al. 1999) to basket configurations of multiple sensors (Schmermund et al. 2003).

Initial ex-vivo studies on carotid endarterectomy specimens demonstrated temperature heterogeneity in plaques which correlated with cell density (Casscells et al. 1996). The temperature had a positive correlation with macrophage density but inverse correlation with smooth muscle-cell density (Madjid et al. 2002). Furthermore, lipidic areas were found to have higher temperatures than calcific areas (Naghavi et al. 2002).

The first human coronary study found greater temperature heterogeneity in plaque of acute coronary syndrome patients compared to stable angina, independent of lesion severity (Stefanadis et al. 1999). Increase plaque temperatures >0.5°C in patients undergoing PCI were strong predictors of adverse cardiac events when followed up for nearly 18 months (Stefanadis et al. 2001). The same group found strong correlations between serum amyloid A and C-reactive protein (CRP) with increased plaque temperature, strengthening the role of inflammation in plaque vulnerability (Stefanadis et al. 2000).

It has been used successfully in acute myocardial infarction which has resulted in a total occlusion to distinguish the site of culprit plaque (Takumi et al. 2007). They found the site
of maximal temperature to be distal to the most stenotic point, indicative of inflammation and plaque rupture which was confirmed by IVUS.

Currently, the technique requires further validation as it’s uncertain if the small differences in temperature can be attributed solely to vulnerable plaques. Other factors such as inflammatory state, medical conditions and medication may impact on this. Furthermore, in isolation it does not provide a visual image of the plaque and would most likely needed to be used in conjunction with another invasive imaging modality.
1.3.1.7 Near-Infrared Spectroscopy

Near-infrared Spectroscopy (NIRS) is a technique which detects distinct chemical composition of the vessel wall or plaque to establish an index of vulnerability. NIRS emits light (wavelengths of 800 – 2500 nm) and measures the amount that is absorbed and scattered across multiple wavelengths to determine the chemical composition of the tissue (Caplan et al. 2006). Its ability to identify cholesterol in plaques was validated initially in rabbits (Cassis & Lodder 1993) then ex-vivo human aorta (Jaross et al. 1999) and carotids (Wang, J et al. 2002).

The ability to detect TCFA was tested in ex-vivo human aorta with good results for lipid pool, thin cap and presence of inflammatory cells (Moreno et al. 2002). Similarly, in ex-vivo human coronaries, NIRS achieved good receiver operating curve c-statistics for detection of lipid core plaques against histology (Gardner et al. 2008). The initial report of a multicenter in-vivo human coronary trial (SPECTACL) showed that NIRS was safe and spectral data obtained were similar to autopsy specimens (Waxman et al. 2009).

A catheter based device incorporating both IVUS and NIRS has been developed and its first-in-man use has been reported to detect lipid rich plaque (Schultz et al. 2010). The technology is still in its infancy and its routine clinical use is yet to be established.
1.3.2 Non-invasive imaging of atherosclerotic plaque

The assessment of atherosclerotic plaques by non-invasive techniques has been possible due to recent technological improvements in multi-detector CT and MRI. While ultrasound has been used to assess severity of carotid stenosis, peripheral vascular disease and abdominal aortic aneurysm, it provides only limited information on plaque volume and characteristics. Non-invasive ultrasound or echocardiogram is unable to successfully image coronary plaques or bypass grafts for the purpose of plaque assessment.

1.3.2.1 Multi-detector computed tomography (MDCT)

The challenges in imaging the coronary vessels were to overcome cardiac motion and be of sufficient quality to reliably detect a lesion. Initial CT technology involved fixed detectors in the form of ‘electron beam CT’ which provided very good temporal resolution (50 – 100 ms) but poor spatial resolutions and long scan times (>20 s). They were used primarily for detection and quantification of coronary calcium (Agatston et al. 1990) and it was not until the 1990s that the first coronary CT angiogram was described (Achenbach et al. 1998).

The rapid development of MDCT with increasing number of finer detectors and faster gantry rotation times have resulted in improved spatial and temporal resolutions to provide good images of the coronaries during diastole when there is least motion (Table 1.3.2).
Table 1.3.2. Timeline and improvements in MDCT.

<table>
<thead>
<tr>
<th>Detectors</th>
<th>Year introduced</th>
<th>Temporal resolution</th>
<th>Detector width</th>
<th>Coverage (z-axis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1999</td>
<td>400 ms</td>
<td>1.0 – 1.5 mm</td>
<td>0.6 cm</td>
</tr>
<tr>
<td>16</td>
<td>2001</td>
<td>190 – 250 ms</td>
<td>0.5 – 0.75 mm</td>
<td>1.2 cm</td>
</tr>
<tr>
<td>64</td>
<td>2004</td>
<td>165 -200 ms</td>
<td>0.5 – 0.625 mm</td>
<td>2.9 - 4 cm</td>
</tr>
<tr>
<td>64 x 2 DSCT</td>
<td>2005</td>
<td>83 ms</td>
<td>0.6 mm</td>
<td>2.9 cm</td>
</tr>
<tr>
<td>256</td>
<td>2007</td>
<td>135 ms</td>
<td>0.625 mm</td>
<td>8 cm</td>
</tr>
<tr>
<td>320</td>
<td>2007</td>
<td>175 ms</td>
<td>0.5 mm</td>
<td>16 cm</td>
</tr>
<tr>
<td>128 x 2 DSCT</td>
<td>2008</td>
<td>75 ms</td>
<td>0.6 mm</td>
<td>4 cm*</td>
</tr>
</tbody>
</table>

DSCT denotes dual source CT. *Coverage of whole heart possible with high-pitch protocol

The majority of coronary CT angiograms performed today are done on 64-detector systems which require a stable regular heart rate of <65 beats per minute, breath hold of <10 seconds and about 5 heart beats to image the entire heart. Due to these limitations, there may be potential artefacts introduced by motion, breathing, ectopic beats or arrhythmias.

The 16-detector CT systems were the first practical systems for coronary imaging as they had similar spatial and temporal resolutions to current 64-MDCT systems. However, due to the limited number of detectors, hence coverage, patients had to breath-hold for 15-20 seconds and image across many heart beats which increased the likelihood of artefacts.

The spatial resolution of a CT system was historically limited by the detector width. An axial CT image is made up of three dimensional voxels which have an in-plane and usually worse through-plane (z-axis) resolution. A typical 16 detector system may have 0.6 x 0.6 mm in plane resolution with a z resolution of 0.75mm (Sensation 16, Siemens, Germany).

By employing a flying focal spot to double the samples across the scan field of view, we can collect information from the same physical detectors from slightly different viewpoints.
resulting in better spatial resolution (Flohr et al. 2005). When this is applied in the z-axis, we could improve on the spatial resolution eg. a detector width of 0.6mm may result in an isotropic resolution of 0.3mm from oversampling eg. Somatom Definition AS+, Siemens Medical, Germany.

Recently, one manufacturer has introduced a high-definition scanner with an in-plane spatial resolution of 0.23 mm with 64-detectors (Discovery CT750 HD, GE Healthcare, USA). This is approaching the spatial resolution of invasive coronary angiography which is 0.20 – 0.25 mm. Utilizing a novel statistical iterative reconstruction algorithm (ASIR), Min and colleagues were able to demonstrate better visualization of coronary stents and greater intraluminal stent area compared with conventional CT systems (Min et al. 2009).

The size of vessel studied has an impact on the diagnostic accuracy of stenosis. Many studies involving 16-MDCT excluded vessels <1.5 mm from analysis. There is a tendency to overestimate lesion severity, especially if there is calcium involved causing a blooming artefact (Hoffmann, MH et al. 2005). Despite the improvements of 64-MDCT, the image quality of segments >2.0 mm is better than <2.0 mm (Pannu et al. 2006), and proximal better than distal (Meijer et al. 2008; Stein, PD et al. 2008).

Since the introduction of 64-detector systems numerous single-centred studies comparing the accuracy of coronary CTA with traditional coronary angiography for significant stenosis have been published. Most have less than 100 patients and used a binary cut-off of 50% stenosis in their analysis. Meta-analyses of these studies have been performed (Table 1.3.3).
Table 1.3.3. Meta-analyses of native coronary arteries studied with 64-MDCT.

<table>
<thead>
<tr>
<th>Author (Reference)</th>
<th>Analysis reporting</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdulla (Abdulla et al. 2007)</td>
<td>Patients 1251</td>
<td>98%</td>
<td>91%</td>
<td>93%</td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td>Segments 18920</td>
<td>86%</td>
<td>96%</td>
<td>83%</td>
<td>97%</td>
</tr>
<tr>
<td>Mowatt (Mowatt et al. 2008)</td>
<td>Patients 1286</td>
<td>99%</td>
<td>89%</td>
<td>93%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Segments 14199</td>
<td>90%</td>
<td>97%</td>
<td>76%</td>
<td>99%</td>
</tr>
<tr>
<td>Stein (Stein, PD et al. 2008)</td>
<td>Patients 2045</td>
<td>98%</td>
<td>88%</td>
<td>93%</td>
<td>96%</td>
</tr>
<tr>
<td></td>
<td>Segments 27099</td>
<td>90%</td>
<td>96%</td>
<td>73%</td>
<td>99%</td>
</tr>
</tbody>
</table>

PPV denotes positive predictive value; NPV, negative predictive value.

From these pooled results, it is clear that coronary CTA has excellent negative predictive value but somewhat variable positive predictive value. Therein lays its strength as a tool to rule out significant coronary artery disease. The lower sensitivity and higher specificity of the analysis by segment are expected as patients are defined as having CAD if any segments are positive for significant disease. The percentage of non-evaluable segments range from 4-8%. The left main has the best sensitivity (91%-100%) and specificity (100%) while the mid right coronary had the worst (81% and 95% respectively) (Meijer et al. 2008; Stein, PD et al. 2008).

The prevalence of CAD in these meta-analyses were high, around 60%, which will impact on the predictive values. Meijboom et al, studied the accuracy of 64-MDCT in symptomatic patients with low (13%), medium (53%) and high (87%) estimated pre-test probability of CAD and found 100% NPV in the low to intermediate group but 89% in the high group (Meijboom et al. 2007).
In recent times, results from multi-centre and sometimes multi-vendor validation trials of 64-MDCT have been published (Table 1.3.4). The CORE64 trial was a highly anticipated single vendor, international multi-centre trial of 291 patients comparing 64-MDCT to conventional angiography (Miller et al. 2008). They reported almost 100% of segments were evaluable but excluded patients with high calcium scores (>600) and vessels <1.5 mm. The poorer NPV of 83% was seen as reflective of the high prevalence of CAD (56%) in their population. Although they found that MDCT could accurately identify the presence of significant CAD and those requiring subsequent revascularization, they concluded that coronary CTA cannot replace conventional angiography at present.

Table 1.3.4. Prospective multi-centre validation trials of 64-MDCT

<table>
<thead>
<tr>
<th>Author</th>
<th>Analysis reporting</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller (Miller et al. 2008)</td>
<td>Patients</td>
<td>291</td>
<td>85%</td>
<td>90%</td>
<td>91%</td>
</tr>
<tr>
<td>Meijboom (Meijboom et al. 2008)</td>
<td>Patients</td>
<td>360</td>
<td>99%</td>
<td>64%</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td>Segment</td>
<td>5297</td>
<td>88%</td>
<td>90%</td>
<td>47%</td>
</tr>
<tr>
<td>Budoff (Budoff et al. 2008)</td>
<td>Stenosis &gt;50%</td>
<td>95%</td>
<td>83%</td>
<td>64%</td>
<td>99%</td>
</tr>
<tr>
<td></td>
<td>Stenosis &gt;70%</td>
<td>94%</td>
<td>83%</td>
<td>48%</td>
<td>99%</td>
</tr>
</tbody>
</table>

PPV denotes positive predictive value; NPV, negative predictive value.

The assessment of CABG has always been much easier that native coronaries due to their relative larger size and immobility. Even with 4-detector (Lau et al. 2006) and 16-detector systems (Martuscelli et al. 2004; Schlosser et al. 2004) the image quality was good but evaluation of distal anastomosis and native vessels have been challenging. With 64-MDCT, 100% of grafts were evaluable with excellent NPV (100%) and PPV (92%) (Ropers et al.
2006). However, the native coronaries are often extremely disease and calcified which resulted in NPV (96%) and PPV of only 44%.

There have been a number of studies comparing 16 and 64-detector CT systems to IVUS in terms of accuracy in measuring stenosis severity, lumen size and plaque area. One of the first studies using 16-detector CT for plaque volume by Achenbach et al, showed a good correlation of $r = 0.8; p < 0.001$ when compared to IVUS (Achenbach et al. 2004). However, the Bland-Altman analysis found that CT significantly underestimated the plaque volume per segment ($24 \pm 35\text{mm}^3$ v $43 \pm 60\text{mm}^3$) which represents a mean difference of approximately 44%. In a subsequent study, the same group found a very small overestimation by CT of plaque area compare to IVUS (Moselewski et al. 2004). They explained that this difference could be partially due to the window widths and levels setting on CT images used for analysis. They used only 1 empirical setting (500 HU window width, 150 HU window level) and these settings have been found to impact on the dimensions of objects displayed (Funabashi et al. 2003; Lu et al. 2001).

**Radiation**

As a technique which utilizes radiation, MDCT needs to be used judiciously rather than at a population screening level. The amount of radiation delivered to the patient depends on a number of factors such as patient size, gender, distance covered and scanning protocols. The traditional method of scanning is called ‘retrospective scanning’, where radiation is delivered throughout the cardiac cycle. ECG-gated dose modulation is a setting which decreases radiation during systole resulting in 25-40% lower radiation for both men (8 mSv) and women (12 mSv) (Hausleiter et al. 2009; Mowatt et al. 2008).
Another major step in reducing radiation is lowering the power setting of the scanner (from 120 to 100 kV) when the patient is less than 85 kg or has a body mass index < 30 (Raff et al. 2009). This technique achieves the same diagnostic quality images with up to 60% less radiation but has not been commonly used due in part to ignorance of the diagnostic facilities (Hausleiter et al. 2009; Raff et al. 2009).

A recent breakthrough is the ‘prospective scanning’ technique, which delivers radiation only during a very short period in diastole. The radiation reduction is up to 80%, with doses of 2-5 mSv, which is lower than typical invasive coronary angiograms and stress nuclear scans (Table 1.3.5) (Einstein et al. 2007; Hausleiter et al. 2009). However, patients must have stable, low heart rates (<60 bpm) without ectopy or heart rate variability because there is little margin for error.

**Table 1.3.5.** Comparison of average radiation

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Radiation Dose (mSv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual background radiation (USA)</td>
<td>3 mSv</td>
</tr>
<tr>
<td>Chest X-ray (posteroanterior only)</td>
<td>0.02 mSv</td>
</tr>
<tr>
<td>Invasive coronary angiography</td>
<td>7 mSv</td>
</tr>
<tr>
<td>Calcium scoring on cardiac CT</td>
<td>2 mSv</td>
</tr>
<tr>
<td>CTCA – prospective (120 kV)</td>
<td>2 – 5 mSv</td>
</tr>
<tr>
<td>CTCA – retrospective (120 kV)</td>
<td>12 mSv</td>
</tr>
<tr>
<td>Stress nuclear scan (Technitium-99m sestamibi)</td>
<td>11 mSv</td>
</tr>
<tr>
<td>Stress nuclear scan (Thallium-201)</td>
<td>22 mSv</td>
</tr>
</tbody>
</table>

mSv denotes millisievert; CTCA denotes coronary CT angiogram; kV denotes kilovolt;
1.3.2.1.1 Hounsfield Units and Window Settings

CT values or Hounsfield Units (HU) are assigned to each pixel of data which represents the x-ray attenuation of the tissue. The image data is 12 bits which has $2^{12}$ or 4096 shades of gray which ranges from -1024 HU to +3072 HU and water is assigned the value of 0 (Hounsfield 1980). Traditionally, calcium has a value above 130 HU while compact bone is from 250 to 1000 HU; metallic foreign bodies are usually >1000 HU. The windows ‘width’ and ‘level’ settings are selected to allow optimum viewing of certain organs or tissues (Figure 1.3.1). For example, if the level is set at 200 and the width is 800, this means that tissues with values from -200 to +600 are ‘visible’ and is represented with various shades of gray. This is a typical setting used for assessment of coronary CT angiography. If one was to reduce the width down to just 1 then the image will essentially be pure black or white on either side of 200HU. This would allow a sharp demarcation of the vessel lumen from the vessel wall provided one was certain that the contrast in the lumen was greater than 200HU. However, if one was to set a very wide window width, eg. 2000HU, the resultant picture would be lacking in contrast. This would make discrimination of soft tissue difficult but can useful for minimizing the blooming artefact caused by calcium.
Figure 1.3.1. A: Typical window width (W) and level (L) used for coronary CTA examination. There is a large mixed plaque in the proximal left anterior descending artery (white arrow). Ao – aorta; RV – right ventricle. B: This is a ‘lung window’ setting where the level is set very low eg. -500 to visualize lung parenchyma. In this case the width is 1500 which results in any tissue >250 HU to appear bright white. C: The width is only 1 resulting in all objects above 200 HU to be white and below 200 HU to be black. D: Very wide window width resulting in poor contrast image but allowing better visualization of calcium in the mixed plaque.

The window settings can affect the apparent size of vessel lumen and plaque, especially when there is calcification adjacent to lumen or variability in the contrast opacification due to severe stenosis or reduced flow. Individually adapted CT window settings for a
particular segment of artery was superior to a universal setting for CT measurements of lumen and plaque (Funabashi et al. 2003). This was particularly important when used to quantify coronary plaque on 64-detector CT when compared to IVUS (Leber et al. 2005). In this particular study, Leber et al. first measured the HU of the contrast in the lumen in a given cross section of the vessel. In order to trace the lumen area, the window level was set at 65% of the density of the contrast in the lumen and the width was reduce to 1, thereby producing a black and white image. For outer vessel wall area measurements, the level remained the same at 65% but the width was increased to 155% of the original lumen HU intensity. Using this method, they found a good correlation with IVUS in quantifying the plaque area ($r = 0.73; p=0.03$).

In assessing carotid artery stenosis on CT, the studies have varied widely in the window settings but a typical setting would be 700HU and 200HU for window width and level, respectively (Saba, Sanfilippo, et al. 2007). The topic of carotid plaque imaging with CT is discussed further in the section under carotid artery stenosis.

1.3.2.1.2 Discriminating Plaque Types on MDCT

The ability of MDCT to directly visualize coronary atherosclerotic plaque holds great potential beyond mere stenosis measurements. Similar to IVUS, one could quantify plaque volume, derive remodelling and eccentricity indices which may be clinically relevant (Achenbach et al. 2004; Hoffmann, U et al. 2006; Tanaka, Shimada, et al. 2008). In addition, there are high hopes for MDCT to discriminate different plaque components, especially lipid-rich from fibrous plaques as this may lead to identification of ‘high risk’ plaques.
The ability to detect calcium in plaques has been well established in numerous studies of coronary artery calcium (CAC) scores and MDCT (Greenland et al. 2007). In fact, CT has such high sensitivity to calcium, one needs to be mindful of ‘blooming’ artefacts which may make calcium deposits appear larger and obscure adjacent plaque or lumen (Hecht & Bhatti 2008; Sarwar et al. 2008).

Attempts to distinguish lipid-rich from fibrous plaques, based on the former having lower mean HU density, have been met with variable success. There is significant overlap in HU density between lipidic and fibrous plaques which have been identified on CT when compared with histology and IVUS (Ferencik et al. 2006; Leber et al. 2004; Motoyama, Kondo, Anno, et al. 2007). However, Leber et al. found hypoechoic plaques on IVUS (likely lipid-rich) had mean CT density of $49\pm22$ HU while hyperechoic plaques (likely fibrous) were $91\pm22$ HU; calcified plaques were $391\pm156$ HU (Leber et al. 2004). In a recent study using dual-source CT and IVUS, significant overlap between fibrous and lipidic plaques were once again confirmed (Marwan et al. 2011). However, the authors used software to analyse the distribution of HU within each non-calcified plaque and generated a histogram. They determined if greater than 5.5% of the pixels of any given plaque were $<30$ HU, the likelihood of it being lipidic was high: sensitivity 95%; specificity 80%; AUC 0.9 (95% CI 0.7-1.0).
1.3.2.1.3 Plaque features of ACS patients

In attempts to demonstrate or characterize ‘high-risk’ plaques, MDCT scans have been performed in patients soon after ACS or AMI, often in comparison with stable angina patients. Patients with ACS had plaques which were more positively remodelled, lower in HU density and contain some spotty calcification compared with those with stable angina (Hoffmann, U et al. 2006; Motoyama, Kondo, Sarai, et al. 2007). Furthermore, recent studies found culprit lesions had greater remodelling index, lower HU density and larger volume compared to non-culprit lesions of patients with ACS (Kitagawa et al. 2009; Pflederer et al. 2010).

ACS patients also had greater number of plaques compared with stable angina patients with positive myocardial perfusion imaging (MPI), which in turn had more plaques than angina patients with negative MPI (Sato et al. 2010). In a study of 1,059 patients with a mean follow up of 27 months, 22% of patients with positive remodelling and low attenuation plaques (<30HU) on baseline MDCT subsequently developed ACS (Motoyama et al. 2009). However, only 3.7% of patients with either features and 0.4% of patient with neither features developed ACS. These studies add weight to the hypothesis that high risk plaques tend to be lipid-rich with positive remodelling – features which can be identified on MDCT.
1.3.2.2  

**Magnetic Resonance Imaging**

MRI is a unique technology which allows noninvasive visualization of cardiovascular anatomy without ionizing radiation. However, there are some limitations including long imaging time, confined space and is contra-indicated in patients with certain metallic implants. It has the potential to provide information on cardiac anatomy, function (myocardial and valvular), perfusion and metabolism.

Unlike MDCT, the acquisition of MRI images and their subsequent quality is largely operator dependent. The study typically starts with a few scout images which the operator can then plan other images. The operator selects where a 2-D slice of an image will cut through and can form a volumetric slab by shifting the slice position by a few millimetres in the same plane. Therefore, to acquire images of atherosclerotic plaque, one has to start at one end of the vessel and move along its axis acquiring each 2-D slice (which takes several seconds) until the entire lesion is covered.

1.3.2.2.1  

**MRI Basics**

While the exact physics of MRI techniques are beyond the scope of this chapter, it is worthwhile to understand some basic concepts and key imaging techniques used. A patient is partially magnetized when placed in a MRI scanner due to the application of a radiofrequency pulse. The nuclei of atoms with odd number of protons are most susceptible and are said to be MR active. In humans, the hydrogen nucleus is targeted as it
is abundant in water and its solitary proton gives it a large magnetic moment (Westbrook et al. 2005).

Image acquisition involves subjecting a patient to subsequent magnetic fields which are perpendicular to previous fields, resulting in a vector of magnetisation within the target tissues. This generates a small voltage in the receiver coil and is converted to an MR signal. The characteristics of these signals are dependent on MR parameters and the tissues themselves which allows for accurate differentiation of various components of the body.

In relation to cardiovascular applications, there are 3 main MR pulse sequences:

1. Spin echo imaging
2. Gradient echo imaging
3. Steady-state free precession imaging (SSFP)

Spin echo imaging is the main sequence used for static structures (eg. vascular wall, myocardial wall thickness and intracardiac thrombus) and can provide excellent discrimination between various soft tissues. In this technique, fast flowing blood through the image plane appears dark (so called ‘black blood’ imaging). By manipulating different parameters such as the repetition time (TR) and echo time (TE), different contrast weighting images can be formed eg. T1, T2, proton density (PDW).

T1 weighted imaging is usually good for defining anatomy. Fat, haemangioma, slow flowing blood and contrast agents will return a high signal intensity and appear bright. Bone, calcification, infarction and fast flowing blood will have very low or no signal, thereby appearing dark.
T2 weighted imaging is traditionally good for defining pathology due to increase fluid content in tissues. High signal intensity is found in tissues with oedema, infection, inflammation and slow flowing blood. Low signals are found in calcification, air, haemosiderin and fast flowing blood.

The contrast in a proton density weighted image is predominantly due to the differences in the proton density of the tissues. Tissues with low proton density (eg. cortical bone and air) will be dark due to low number of protons contributing to transverse magnetization.

Gradient echo imaging takes significant shorter time to acquire than spin echo imaging and is used in cardiac cine imaging to demonstrate myocardial function and vascular lumen angiography. The flowing blood appears white without needing any contrast (so called ‘bright blood’ imaging). It can acquire 10-15 images per second and allow for real-time imaging. However, there is poorer discrimination of various tissue contrasts when compared to spin echo imaging.

Steady state free precession (SSFP) imaging is an improvement of gradient echo imaging, with even faster imaging times. Blood appears very bright which improves contrast between it and surrounding tissues like myocardium. It is used in cine imaging for assessment of cardiac function, myocardial perfusion and viability assessment.
1.3.2.2 Coronary MRA

It has been 20 years since initial reports of the ability to image the coronaries with breath-holds of up to 18s (Edelman et al. 1991). Often no exogenous contrast is needed for coronary MRA. Using bright blood imaging sequences, image quality was good and compared favourably to invasive angiogram with PPV of 85% and NPV of 95% (Manning et al. 1993). However, the resolution obtainable was approximately 1mm and was not sufficient for routine clinical use. Development of MR ‘navigator echo’ allow free breathing acquisition of coronaries over many minutes by reducing motion artefacts by analysing data when the diaphragm or heart were in a small range of positions (Botnar et al. 1999). With the advent of black-blood imaging and improvement in spatial resolutions to 0.46mm, visualization of the vessel wall in addition to lumen was possible (Fayad et al. 2000). A direct comparison of bright and black blood techniques were made in a small trial (Maintz et al. 2004). There was no clear advantage to either techniques but black bloods can be difficult to interpret as calcification and motion artefacts may lead to signal attenuation and look black (Stuber et al. 2001).

A landmark multi-centre study compared MRI to invasive angiography in 109 patients using volume-targeted coronary MRA protocol (Kim et al. 2001). This technique is operator dependent, requiring accurate localization of coronaries and only limited access to distal vessels & branches. They reported an overall accuracy of 72% in diagnosing significant CAD but 16% of coronary segments were not evaluable. All patients with left main or triple vessel disease were correctly identified by MRA.
The advent of parallel imaging and steady-state free precession (SSFP), “whole heart MRA” with a single breath-hold became feasible (Weber et al. 2003). This technique was less operator dependent and was found to have an improved overall accuracy of 89% when compared with invasive angiography (Jahnke et al. 2005). A recent study comparing whole heart MRA to volume targeted MRA using exogenous contrast found more visible segments and side branches with the former (Tang et al. 2009). Typical spatial resolutions achieved are 0.7-0.8 mm in plane and 1-3mm through plane (Bluemke et al. 2008).

Initial reports of imaging coronaries using high field 3T systems have been reported (Gharib et al. 2007; Stuber et al. 2002). Compared with traditional 1.5T systems, 3T can offer twice the signal to noise ratio which can theoretically result in a four-fold reduction in scanning time. However, one problem with 3T can be reliable R-wave triggering which may be overcome with sophisticated algorithms or T-wave triggering instead.

The ability of MRI to evaluate CABG has also been reported (Aurigemma et al. 1989; Langerak et al. 2003). Flow through the bypass graft was able to be measured and when incorporated with MRI images yielded high sensitivity (96%) and specificity (92%) in detecting obstructive >70% stenosis (Langerak et al. 2003). However, only 80% of grafts were scanned successfully.

Despite these improvements in MRI technology, coronary MRA has not seen widespread clinical use. This is in part due to limited expert centres and access to MR systems. However, the main factor currently is the competition it faces from MDCT which offers superior spatial resolution, speed, diagnostic accuracy and widespread availability (Bluemke et al. 2008; Schuetz et al. 2010; Schuijf et al. 2006).
1.3.2.3 **Positron Emission Tomography**

Positron Emission Tomography (PET) utilizing fluorine-18-labeled-2-deoxy-D-glucose (FDG) as tracer is a very sensitive nuclear medicine imaging modality used to detect cancer recurrence or metastasis in oncology patients. FDG is a direct surrogate of glucose uptake, hence when taken up by cells is a reflection of their metabolic activity (Shankar et al. 2006). Not only is FDG taken up by tumour cells but also macrophages. Preliminary work has shown that PET imaging is able to identify macrophage rich lesions in both the human carotid artery (Rudd et al. 2002) and rabbit aorta (Zhang et al. 2006). Macrophages appear to play a key role in both risk of plaque rupture and modulation of the plaque’s subsequent thrombogenicity. The largest histological study of symptomatic carotid plaques found that carotid plaques have similar pathology to culprit coronary plaques (Redgrave et al. 2006). Dense plaque inflammation with macrophage infiltration was the feature most strongly associated with plaque cap rupture and time since stroke, suggesting possible causal links between plaque inflammation and plaque instability.

Although PET scan takes approximately 15 minutes there is often 1½ – 2 hours between injection of the radioactive tracer and scan to allow sufficient time for FDG accumulation in the vascular wall and decay in the blood (Rudd et al. 2010). PET imaging by itself has a relatively poor spatial resolution of 3 – 4mm, requiring co-localization with CT or MRI images. In the last few years there have been PET/CT hybrid machines of 4, 16 & 64 detectors which automatically co-register images as they are obtained at the same time. In 2010, a clinical PET/MRI machine (Biograph mMR, Siemens, Germany) has been announced.
In a pilot study, patients with symptomatic carotid stenosis had metabolically active plaques on PET imaging (Figure 1.3.2) as compared to the contralateral carotid artery (Rudd et al. 2002). Histopathology specimens of the active plaques demonstrated a macrophage-rich infiltration at the border of the lipid core and fibrous cap. A further study has demonstrated that $^{18}$FDG uptake on PET imaging can determine the degree of inflammation and macrophage content of carotid plaques in vivo (Tawakol et al. 2006).

![Figure 1.3.2. Images of a carotid artery plaque from PET (left), CT (middle) and a fusion of the two modalities (right). The arrows point to an area of bright orange uptake in PET and corresponding stenosis on CT. The fused image confirms that the uptake occurs in the plaque of the stenosis (Rudd et al. 2002).](image)

Recent carotid studies have found higher signals from plaques with high risk features such as large lipid core on MRI (Silvera et al. 2009), high echolucency on ultrasound (Graebe et al. 2010) and intra-plaque haemorrhage (Kwee et al. 2009).

In a rabbit model of atherosclerosis, FDG-PET was able to show the progression and regression of inflammation in aortic plaques when rabbits were randomized to an atherosclerotic or normal diet after 6 months of being on a high-fat diet (Worthley et al. 2009). A recent randomized control trial in humans has shown the ability of simvastatin (5-
20 mg) to attenuate $^{18}$FDG uptake in aortic and carotid plaques (Tahara et al. 2006).

However, it did not document the degree of carotid stenosis (if any) of the participants which comprised of volunteers for a cancer screening PET scan.

Demonstrating inflammation in the coronary plaques using FDG-PET has been challenging due to poor spatial resolution of PET, motion of coronaries and high metabolic activity of the adjacent myocardium which can drown out signals from the coronaries (Dunphy et al. 2005). There is the added complexity of reconciling the spatial shifts of coronary CTA obtained during breath-holds and PET which are free-breathing. Utilizing a low-carbohydrate, high fat meal the night previous to PET scan and drinking vegetable oil the day of scan, it was possible to suppress myocardial FDG uptake in 63% of patients for demonstrating inflammation in the coronaries (Wykrzykowska et al. 2009). High-fat diets presumably cause the myocardium to metabolize free fatty acids rather than glucose via a myocardial glucose transporter protein (GLUT4) which is absent in plaque macrophages (Rudd et al. 2010). In a recent human study, coronary plaques of patients with ACS have higher FDG signals compared with those of stable angina controls (Rogers, IS et al. 2010).

Beyond pure anatomy, functional imaging such as PET demonstrates the importance of inflammation in the deterioration of high-risk plaques. In future, this inflammatory activity may be an indicator to the temporal relationship between high risk features on anatomy and subsequent rupture or clinical events. It is uncertain how or when this may prove useful in risk stratification of any given patient, but it will most like be used in a very select population perhaps after high-risk anatomical features have been identified by other means.
1.3.3 Conclusions

The identification of vulnerable plaques via imaging continues to challenge us. There are numerous approaches via invasive means, some of which are able to demonstrate features identified on histology. However, none can confidently accomplish the task by itself and combination catheters which marry multiple technologies may increase our success rate in the future.

It is intuitively attractive if we could identify vulnerable plaques with non-invasive techniques. At least for coronary plaques, MDCT has emerged to be the modality with most promise. Advances have reduced the radiation involved while maintaining image quality. However, the spatial resolution is still relatively poor compared to invasive modalities and there are issues with discrimination of non-calcified plaque components. Given the current limitations, MDCT may perhaps serve as an early or intermediate role in future pathways to risk stratification of a ‘vulnerable patient’. At present, none of the non-invasive modalities can reliably identify high risk plaques. It is likely that one may need to adopt a multi-modality approach to solve this puzzle.
1.4 Saphenous Vein Graft Disease

The earliest form of CABG using saphenous veins was performed by Favaloro in 1967 and has been developed subsequently to become a widely used conduit for coronary bypass today (Favaloro 1998). Although the CABG procedures have declined in recent years in Australia due to improved PCI techniques, utilization of SVGs as a conduit for revascularization remains a popular choice (AIHW 2008). However, up to 20% of SVGs occlude in the first year with an annual occlusion rate of 2-5% and approximately 40% are occluded at 10 years (Sabik et al. 2005; Shah, PJ et al. 2003). Of the patent grafts at 10 years, only 50% are free from significant stenosis (Fitzgibbon et al. 1996).

Early graft failure is likely related to surgical technique or poor targets while those which fail after a many months are related to neointimal hyperplasia and later on atherosclerosis (Fitzgibbon et al. 1996; Shuhaiber et al. 2002).

1.4.1 Pathogenesis of SVG disease

The native saphenous vein is more muscular and rich in elastin than other veins (Szilagyi et al. 1973). It has a thin intima consisting of a layer of endothelial cells on a fragmented internal elastic lamina which is poorly defined (Motwani & Topol 1998). The media has three layers of smooth muscle separated by loose connective tissue. The adventitia has smooth muscle cells, elastin fibres and collagen arranged longitudinally. The saphenous vein also has a high dependence on the vasa vasorum for blood supply (Motwani & Topol
This makes SVG dependent on diffusion of blood in the first week after implantation.

It is thought that thrombosis plays a major role in early graft failure, as even with careful harvesting, there is focal endothelial disruption and damage to the media (Roubos et al. 1995). This results in the activation of the clotting cascade and alterations in local and circulating factors influencing homeostasis. During the peri-operative period, there is marked elevation of plasma fibrinogen which favours a prothrombotic response (Moor et al. 1994).

Between the first month and 1 year after surgery, intimal hyperplasia is the predominant disease process in SVGs. Accumulation of smooth muscle cells and extracellular matrix in the intima occurs within 4-6 weeks of surgery. It may reduce the lumen but rarely results in significant stenosis (Chesebro & Fuster 1986). The transient ischaemia and reperfusion which occurs during grafting induces superoxide radical formation which promotes smooth muscle cell proliferation and also reduces anti-proliferative mediators (Holt et al. 1993). There is a substantial loss of the original endothelium and deposition of platelet and fibrin which leads to organizing fibrous tissue, further promoting proliferation of smooth muscles cells (Verrier & Boyle 1996). Finally, there is an acute increase in wall stress cause by implantation of veins into arterial circulation. This results in both increased growth factors and reduction in growth inhibitors, thus promoting intimal hyperplasia (Allaire & Clowes 1997). Incidentally, in an experimental model of vein-to-vein grafting, intimal hyperplasia was absent, suggesting the pathology was a response to the haemodynamic and physiological changes of veins implanted into arterial circulation (Zou et al. 1998).
Atherosclerosis becomes the main cause of SVG disease 1 year post surgery and is markedly increased after 5-7 years of grafting. In patients presenting with ACS post CABG, the culprit lesions were determined to be within SVGs in 70% -85% of cases, often with superimposed thrombus (Chen et al. 1996). The process of atherosclerosis occurs more rapidly in vein grafts and is thought to be due to chronic injury to the endothelium (Verrier & Boyle 1996).

There are subtle differences between atherosclerotic plaques of arterial and vein grafts. The latter tends to be diffuse, concentric and more friable, containing more inflammatory and foam cells (Kalan & Roberts 1990; Neitzel et al. 1986). In animal models, lipid accumulates in vein grafts faster while being broken down slower, thereby contributing to the relatively proatherogenic conditions (Shafi et al. 1987).

Multiple factors contribute to the development of SVG disease including age of graft, smoking, diabetes, hyperlipidaemia and hypertension (Motwani & Topol 1998). Women have worse mortality and morbidity with CABG and poorer graft patency at 2 years (Loop et al. 1983). Morphologically, the diameter of native vessels being grafted >1.5 mm has a good patency of 90% at 1 year compared with 65% if <1.5mm (Roth et al. 1979). It is a commonly held belief that if the bypassed segment was not severely stenosed (>70%), the SVG would likely fail (Roth et al. 1979). However another study using a 50% stenosis cut-off did not find a significant difference in SVG patency (Cosgrove et al. 1981).
1.4.2 Prevention of SVG disease

The prevention of SVG disease is similar to strategies and secondary preventive measures for CAD in general. They revolve around the use of antiplatelet agents, lipid-lowering therapy and cessation of smoking. In a study of 415 patients with 15 years of follow-up, persistent smokers were at greater risk of AMI and reoperation at 1 and 5 years post-surgery compared to those who had quit (Voors et al. 1996). There was a survival advantage at 10 years to those who quit smoking (84% v 68%; p=0.018) in the CASS study (Cavender et al. 1992).

Aspirin use soon after surgery was shown in a meta-analysis to result in a 41% relative risk reduction of SVG occlusion (APT 1994). A large prospective observational study found aspirin to be safe post CABG with reductions in mortality and ischaemic complications in multiple vascular beds (Mangano 2002). The use of dual antiplatelet, clopidogrel in addition to aspirin, has been reported in a number of trials with conflicting results. An initial study of 100 patients showed a trend towards improved patency of radial arteries but not SVGs (Sun et al. 2010). A larger randomized trial of 249 patients found a small improvement in SVG patency (91.6% v 85.7%; p=0.043) at 3 months with dual antiplatelet use (Gao et al. 2010). Both these studies used MDCT to assess graft patency. However, a trial using IVUS and invasive angiography in 113 randomized patients found no difference in SVG intimal hyperplasia and patency at 1 year (Kulik et al. 2010).

Aggressive lipid lowering therapy has been shown to decrease progression of graft atherosclerosis and need for revascularization (Campeau et al. 1999; Post-CABG 1997). The target was to lower LDL cholesterol to less than 2.6 mmol/L using a statin and
cholestyramine if needed. The delay in disease progression was seen in both sexes, across age groups and regardless of risk factors (Campeau et al. 1999). The same investigators found a 30% reduction in need for revascularization and 24% reduction in composite cardiovascular endpoints with intensive lipid-lowering at a later follow-up of 7.5 years (Knatterud et al. 2000). The use of gemfibrozil to elevate low HDL (<1.1 mmol/L) has been shown to result in fewer new lesions in SVGs and delay atherosclerosis of native coronaries (Frick et al. 1997).

**1.4.3 Repeat CABG for SVG disease**

In cases where medical therapy for non-urgent symptomatic SVG lesions is insufficient, the options are usually repeat CABG or PCI.

There is an increased risk of mortality and morbidity with repeat CABG compared to the first operation. The Veterans Administration Cooperative Study reported a 10.3% mortality for repeat CABG compared to 5.8% for first CABG (Peduzzi et al. 1998). This difference was echoed in a subsequent large observational study with mortality rates of 6.1% v 1.5% for repeat and first CABG respectively (Yau et al. 2000). The same study also found a higher rate of perioperative AMI (7.4% v 3.7%) for repeat CABG.

Only a handful of studies have directly compared the outcomes of repeat CABG with PCI. Most of these studies were conducted in the late 1990s to early 2000s when dual antiplatelet use and aggressive statin therapy as secondary prevention was not as common as today. The AWESOME randomized trial of 142 patients found a higher in-hospital
mortality (8% v 0%) in repeat CABG compared with PCI (Morrison et al. 2002). However, there was no difference in survival at 3 years.

In a retrospective observational study from the Cleveland Clinic (n=2191), there was no difference in 30-day mortality between repeat CABG and PCI but there was a greater risk of in-hospital Q wave AMI for repeat CABG (Brener et al. 2006). At 5 years, there was no statistical difference in the adjusted mortality rate but there was a trend in favour of repeat CABG. Predictors of mortality included poor left ventricular ejection fraction, age and diabetes. Another observational study of only diabetic patients (n=1123), found a higher rate of in-hospital mortality (11.2% v 1.6%) for repeat CABG but the 10-year mortality was not different from PCI (Cole et al. 2002).

1.4.4 Percutaneous Coronary Intervention for SVG Disease

The outcomes for PCI in SVG lesions are worse than PCI in native coronaries. In part, this is due to the nature of SVG atherosclerotic plaque being more friable and prone to embolization (Mautner et al. 1992). Furthermore, the absence of side branches in SVGs contributes to a greater risk of stasis and thrombus formation in the event of a distal occlusion (Stone et al. 2005). There are increased mortality rates at 30 days (2.1% v 1%) and 6 months (4.7% v 2.0%) for PCI in SVG compared to native coronaries (Roffi et al. 2002). In the setting of primary PCI, the outcomes are also worse for SVG lesions compared to native coronaries. Distal embolization occurred in over 50% of cases and the in-hospital mortality was 19% for SVG cases (Watson et al. 1999).
Stenting

PCI has largely replaced PTCA for treatment of SVG stenosis as there is better procedural success rate (92% v 69%) and lower morbidity (death, AMI, revascularization) as reported in the randomized SAVED trial (Savage et al. 1997). Observational data also showed less angiographic restenosis in SVG treated by PCI (29% v 43%) compared to PTCA (Keeley et al. 2001). These studies have been conducted using bare metal stents (BMS).

The use of drug eluting stents (DES) compared to BMS in SVGs has been evaluated in both observational and randomized trials. The SOS randomized trial which studied paclitaxel-eluting stents found less angiographic restenosis at 12 months (9% v 51%) in comparison with BMS (Brilakis et al. 2009). When followed for median of 1.5 years, there was less need for revascularization but no mortality advantage in using DES. Similarly in the RRISC trial which evaluated sirolimus-eluting stents, there was less in-stent late lumen loss with DES at 6 months (Vermeersch et al. 2006). However, at 32 months they found 11 deaths occurring in the DES group and none in the BMS group (Vermeersch et al. 2007). The recent ISAR-CABG randomized trial of 610 patients found a lower 1-year major adverse cardiac events (MACE) in DES group (15% v 22%, p=0.02), however this was primarily driven by target vessel revascularization and there was no difference in mortality (Mehilli et al. 2011). Currently, BMS is still the predominant stent type used for SVG intervention, in part due to the relatively larger size of SVGs compared to native coronaries. However the use of DES will likely increase as further data on restenosis and revascularization from other ongoing randomized trials becomes available.
The high peri-procedural complications involved with SVG-PCI has been partly attributed to embolization of plaque material. A possible solution to isolating the friable and degenerative SVG plaque during PCI is the use of covered stents. The polymer membrane cover gaps between the struts in the hope of reducing complications related to extrusion and embolization of plaque material. However, randomized trials such as RECOVERS and STING which compared covered stents with bare metal stents failed to show a reduction in restenosis and actually had a higher incidence of myocardial infarction (Schachinger et al. 2003; Stankovic et al. 2003). Similarly, the 5-year follow-up of the multi-centre randomized BARRICADE trial showed a higher target vessel failure of 68.3% vs 51.8% in the covered stent patients (Stone, Goldberg, et al. 2011). Therefore, covered stents have largely fallen out of favour for SVG-PCI.

1.4.5. Prevention and Treatment of No-Reflow

No-reflow is a condition whereby there is inadequate myocardial perfusion without angiographic obstruction of the coronary artery supplying that territory (Niccoli et al. 2010). It is independently associated with increased morbidity and poorer prognosis (Gibson et al. 1999). Once established, no-reflow can be difficult to treat and prevention of its occurrence seems prudent (Niccoli et al. 2010).

The incidence of no-reflow can be as high as 10-15% in SVG interventions (Kaplan et al. 1996). The mechanisms of no-reflow are complex and multi-factorial in nature, involving processes of distal embolization, susceptibility of myocardium to injury, ischaemic injury and reperfusion injury (Niccoli et al. 2009). It is thought the disturbance of vasoregulatory pathways and subsequent microvascular spasm play a central role in no-reflow
(Reffelmann & Kloner 2006). Furthermore, a study of embolic protection device found the aspirate of debris laden blood from SVG-PCI to contain vasoconstrictor substances (Leineweber et al. 2006). It is therefore not surprising that pharmacological strategies have been focused on vasodilators and to a lesser extent, anti-platelet therapy.

A number of vasodilators have been tried in the prevention and treatment of no-reflow. Intracoronary nitroglycerin which acts predominantly on the epicardial vessels has been shown to be of little beneficial while verapamil seems to re-establish normal flow (TIMI 3) in up to 88% of cases (Kaplan et al. 1996). Pre-treatment of SVG-PCI with intracoronary verapamil was found to result in better flow as judged by TIMI frame counts, TIMI myocardial perfusion grades and a tendency towards a reduction in no-reflow incidence (Michaels et al. 2002). Intracoronary adenosine which has vasodilatory and anti-platelet (activation & aggregation) properties has been shown to improve TIMI flow in SVG when given in multiple large doses (Fischell et al. 1998). However, prophylactic administration does not reduce the risk of no-reflow in SVG-PCI (Sdringola et al. 2000). In a small study of 19 patients with no-reflow (9 SVG cases), nitroprusside was used as treatment with significant improvement in angiographic flow and blood flow velocity (Hillegass et al. 2001).

In the native coronaries, platelet activation and thrombosis play significant roles in no-reflow, especially in the setting of STEMI. Trials utilizing glycoprotein IIb/IIIa inhibitors in STEMI patients undergoing PCI have shown improved morbidity and mortality (Montalescot et al. 2007). However, the efficacy of GP IIb/IIIa inhibitors in routine SVG-PCI has been disappointing. In a pooled analysis of five randomized trials, there was no benefit in the use of GP IIb/IIIa inhibitors over placebo for 30-day endpoints of death,
myocardial infarction and revascularization (Roffi et al. 2002). However, its use in conjunction with a distal embolic protection device resulted in less no-reflow, distal embolization and abrupt closure, as observed in a substudy of the FIRE trial (Jonas et al. 2006).

Mechanical strategies to remove or debulk the SVG atheroma via various atherectomy strategies have been disappointing. The CAVEAT II randomized trial of directional atherectomy found an increased rate of distal embolization and a trend towards development of myocardial infarction (Holmes et al. 1995). Similarly, the transluminal extraction catheter which uses cutting blades and suction catheter experienced significant distal embolization (Safian et al. 1994). The use of rotational atherectomy in SVG is currently contraindicated by the manufacturer (Rotablator, Boston Scientific, Natick, USA) due to high likelihood of embolic complications but limited studies of its use in hard, calcific and ostial SVG lesions have been reported (Don et al. 2009; Thomas, WJ et al. 2000).
1.4.6 Embolic Protection Devices

Embolic protection devices (EPD) are designed to trap and remove debris which may be dislodged during PCI, in the hope of preventing complications such myocardial infarction or no-reflow. The two main types of devices are distal devices which usually involve a mesh filter or occlusion balloon, and proximal devices which use an occlusion balloon. In general, devices which employ an occlusion balloon may cause ischaemia as they stop the antegrade flow of blood. In contrast, a filter device allows continuous perfusion but may allow particles smaller than their pore size through as well as soluble vasoactive substance which may impact on the distal microvasculature.

1.4.6.1 Distal Protection Devices

These devices usually involve crossing the lesion with the provided angioplasty wire and somewhat bulky device which has the potential to dislodge plaque debris before any protection is in place. They also require a distal landing zone that is relatively free from disease for the occlusion balloon or filter basket to be placed. Therefore, they cannot be used for very distal or anastomotic lesions. While the ease of use varies between devices, the filter based devices tend to be simpler and easier to use.

The first device to be assessed in a randomized trial was the PercuSurge GuardWire (Medtronic, Minneapolis, USA) which consists of a 0.014” wire, distal occlusion balloon and an aspiration catheter. The SAFER trial involved 801 patients and showed there were
significant reductions in AMI (8.6% v 14.7%; p = 0.008), 30-day MACE (9.6% v 16.5%; p = 0.004) and no-reflow (3% v 9%; p = 0.001) in the use of PercuSurge GuardWire (Baim et al. 2002). There was no difference in mortality. The disadvantages of this device were the risk of ischaemia with balloon occlusion, limited visualization for accurate stent placement and complete evacuation of static blood column.

The FilterWire EX (Boston Scientific, Natick, USA) is a distal filter system consisting of a 110 micron pore mesh that springs open after deployment to catch debris while allowing continuous blood flow (Figure 1.4.1). It was assessed in a randomized trial (FIRE) which showed similar results when compared to PercuSurge GuardWire for 30-day composite endpoint of death, myocardial infarction and target vessel revascularization (9.9% v 11.6%; p = 0.53) (Stone et al. 2003). The second generation device, FilterWire EZ, had a lower profile, improved apposition to SVG and smaller pore size of 100 microns. This was found to have much lower 30-day MACE of 3.8% in the BLAZE II registry, but results have so far been in abstract form only (Fiorentino et al. 2008).

![Figure 1.4.1 Two used FilterWires with particulate matter captured.](image-url)
Studies of subsequent devices have yielded similar clinical results to SAFER and FIRE trials. The SpiderRx (ev3, Plymouth, Minnesota) is a nitinol filter device which can be inserted over any conventional 0.014” angioplasty wire, thereby allowing the operator to use a wire which he/she is most familiar with to pass the lesion. It was found to be non-inferior to both GuardWire and FilterWire in the randomized SPIDER trial and subsequently gained U.S. Food and Drug Administration approval (Dixon 2005). Another device is the TriActiv System (Kensey Nash Corp, Exton, Pennsylvania) which uses a distal occlusion balloon and an infusion flush / aspiration system to remove debris. In the PRIDE trial it was non-inferior to GuardWire and FilterWire but showed increased risk of bleeding requiring transfusion (7.7% v 3.5%; p = 0.02) due to vascular complications and requirement for larger catheters (Carrozza et al. 2005).

### 1.4.6.2 Proximal Protection Devices

Currently, there is only one proximal protection device approved for clinical use – the Proxis embolic protection system (St Jude Medical, Maple Groves, Minnesota). It uses a proximal balloon to stop blood flow in the SVG and creates a static column of blood, establishing protection prior to crossing the lesion. The PCI can be carried out using an angioplasty wire of choice and the column of blood containing debris, aspirated before blood deflation. The PROXIMAL trial showed non-inferiority to GuardWire or FilterWire for 30-day composite endpoint of death, MI and revascularization (10% control v 9.2% test; p=0.78) (Mauri et al. 2007).
1.4.6.3  Efficacy of Embolic Protection Devices

The efficacy of embolic protection devices depend on the design, proper use to ensure correct apposition / occlusion and burden of disease which can sometimes overwhelm a filter system. Indeed, if there are copious amounts of large debris obstructing a filter device, a condition of ‘filter no-reflow’ can occur (Porto et al. 2006). Distal embolization occurs in the majority of SVG-PCI and up to 90% of devices retrieved contain some debris (Grube et al. 2002). The various trials of EPD demonstrated that the use of EPD resulted in a 40-50% reduction of adverse events (Baim et al. 2002; Stone et al. 2003). This reduction is largely due to peri-procedural myocardial infarction and EPDs do not reduce mortality per se.

Initial analysis of debris retrieved using balloon occlusion device suggested that 80% of debris was <96 µm in size (Grube et al. 2002). Given that the pore size of most filter devices were 100-110 µm, one would expect that filter devices would perform poorly compared to balloon occlusion devices. A study comparing the various EPDs found the particulate matter captured by balloon occlusion or filter devices were very similar in size (Rogers, C et al. 2004). Particulate size ranged from <56 µm to > 2000 µm, with more than 85% of particles being <96 µm in size captured by both types of devices. A possible explanation could be the adherence of debris to the filter surface forming an aggregate with platelets, fibrin and other material to functionally reduce the pore size. Simple histological analysis indicated that thrombus alone was present in 49% of EPDs and plaque elements were found in additional 38% of EPDs (Rogers, C et al. 2004).
Since 2005, the AHA/ACC guidelines on PCI has given a Class I recommendation that EPDs be used in SVG interventions whenever possible (Levine et al. 2011). However, a report from the U.S. National Cardiovascular Data Registry showed that EPDs were underutilized with 41% of hospitals using EPDs in <10% of cases and 19% of hospitals not using them at all (Mehta, SK et al. 2007).

1.4.7 Predictors of Adverse Events

A major concern of SVG-PCI is the risk of peri-procedural myocardial infarction which traditionally can be measured by elevated myocardial creatinine-kinase (CK-MB). The incidence has been reported to be 15-47% depending on the cut-offs used (Baim et al. 2002; Hong, Mehran, et al. 1999). In a large study of SVG-PCI, there were significant difference in the 1-year mortality (4.8%, 6.5% and 11.7%) between normal CK-MB, mild elevation of 1-5 times normal and major elevation of >5 times normal CK-MB, respectively (Hong, Mehran, et al. 1999). They also found that major elevation of CK-MB was the strongest independent predictor of late mortality in patients undergoing SVG-PCI.

It has been found that markers of increase plaque burden are associated with increased adverse events. Predictors of 30-day MACE post SVG-PCI include lesion length, SVG angiographic degeneration score and estimated plaque volume based angiographic measurements (Coolong et al. 2008; Giugliano et al. 2005; Kirtane et al. 2008).
1.4.8 Conclusions

Saphenous vein grafts remain popular as a conduit in CABG. However, interventional cardiologists are increasingly faced with difficult and often high risk PCIs as SVGs degenerate and occlude. The rate of peri-procedural myocardial infarction and complications are approximately doubled compared to native coronaries, due in part to the high rate of plaque embolization. The use of embolic protection devices has significantly reduced but not eliminated this risk. Furthermore, the problem of no-reflow can often be difficult to prevent and treat. Saphenous vein graft intervention remains highly challenging and further research into methods for risk-stratifying those who will benefit from intervention is needed.
1.5 CAROTID ARTERY STENOSIS

Extracranial carotid artery disease accounts for approximately 20% of ischaemic strokes (Petty et al. 1999). The risk of subsequent strokes in patients with carotid stenosis is highest in the weeks after onset of transient ischaemic attacks (TIAs), and the risk declines rapidly thereafter (Lovett et al. 2003; Rothwell et al. 2004). Carotid imaging performed within this short window period could define the severity of stenosis, and facilitate decision for effective stroke prevention either by medical management or by revascularisation.

Intra-arterial digital subtraction angiography remains the historical gold standard, but luminal stenosis is now predominantly assessed with non-invasive modalities either alone or in combination: carotid duplex ultrasound, magnetic resonance angiography (MRA) and computed tomographic angiography (CTA). The predominant method of measuring stenosis severity in Australia is in accordance with the North American Symptomatic Carotid Endarterectomy Trial (NASCET) by comparing lumen diameter at the most stenotic portion of the vessel to the lumen diameter of the healthy internal carotid artery distal to the stenosis (NASCET 1991). Other methods include The European Carotid Surgery Trial (ECST) and the common carotid method (ECST 1991; Rothwell et al. 1994). Based on NASCET criteria, the consensus on severe carotid stenosis is 70-99% and moderate stenosis is between 50-69% (Bates et al. 2007; Furie et al. 2010).

Whilst we recognized the importance of stenosis severity, several recent studies have turned our attention to morphological parameters which may further refine our
management. These include the type of plaque (lipidic, fibrous or calcific), plaque ulceration, thin or fissured fibrous cap and plaque haemorrhage (Eliasziw et al. 1994; Nandalur et al. 2005; Ouhlous et al. 2005).

**1.5.1 Ultrasound**

Carotid Doppler ultrasound (CDUS) is an inexpensive, portable, non-invasive and safe diagnostic method. It provides flow dynamics, localisation and extent of stenosis. It is reliable in the hands of experienced reporters, with sensitivities of 85-92% and specificities of 77-89% for severe (70-99%) carotid stenosis in a meta-analysis comparing CDUS to intra-arterial cerebral angiography (Wardlaw et al. 2006). It is less precise in determining stenoses of less than 69 percent compared with 70 percent or greater (Carroll 1991; Sabeti et al. 2004; Tsuruda et al. 1991).

CDUS has limited capability in providing information on plaque composition. Echolucent plaques, presuming higher lipid content, were found to significantly carry a higher risk of ischemic events compared to those echogenic plaques (Mathiesen et al. 2001). Diagnostic errors could arise due to operator variability (Mathiesen et al. 2000; Mikkonen et al. 1996), inter-hospital variability (Kuntz et al. 1997), artefacts from calcified plaques, tortuous or kinked carotid arteries, and patient body habitus, as well as difficulty in distinguishing subtotal from total occlusion (Perkins et al. 2000). Most centres would not make clinical decisions based solely on the findings of CDUS, rather as a screening test prior to MRA or CTA (Johnston & Goldstein 2001; Nederkoorn, Mali, et al. 2002).
While it is beyond the scope of this section to review the data on carotid intima-media thickness (CIMT), it is mentioned here briefly for completeness. Most measurements of CIMT are made using one B-mode ultrasound technique in the common carotid artery (Pignoli et al. 1986). It has been used in a number of epidemiological studies to correlate CIMT with increased risk of coronary artery disease (Burke, GL et al. 1995; Howard et al. 1993). In a recent review paper on CIMT and cardiovascular risk, the authors (Simon et al. 2010) concluded that:

(i) CIMT was only a modest predictor of coronary artery disease (CAD).
(ii) CIMT added little beyond the traditional risk factors to predicting CAD.
(iii) CIMT was an independent predictor of stroke
(iv) CIMT was inferior to ultrasound assessment of carotid plaque in predicting CAD, as plaque may be more representative of atherosclerosis than CIMT.

Whilst CDUS is widely available, its technical limitations and operator dependence makes it a less than ideal modality in plaque quantification and characterisation of different plaque components.
1.5.2 Computed Tomography

CT angiography allows rapid examination of the vessel with good sensitivity (70-80%) and excellent specificity (91-97%) in luminal assessment of severe stenosis when compared to invasive angiography (Wardlaw et al. 2006). The main drawbacks are ionizing radiation and potential contrast nephropathy in those with renal impairment. Dense circumferential calcification may obscure the lumen and hence affect the accuracy of lumen assessment (Alvarez-Linera et al. 2003), however this may be partially or fully overcome by post processing techniques (Randoux et al. 2001).

However, it is the ability to visualize the vessel wall, including plaque and surrounding structures which show great promise for CT as a modality. In a small study, CT was able to quantify and characterize carotid plaque components in vivo when compared to histology obtained at subsequent endarterectomies (de Weert et al. 2006). Based on different Hounsfield Unit densities, the authors were able to identify lipidic (<60 HU), fibrous or mixed (60-130 HU) and calcific (>130 HU) areas with good correlations and reproducibility. A recent study utilizing ‘dual-source CT’ and programming each source to emit different levels of x-ray energies (dual-energy CT), sensitivities for identifying calcific plaque was 100%, mixed plaque 89% and lipidic plaque 85% when compared to histology (Das et al. 2009). The ability to identify and quantify certain components may be useful as some MRI studies have demonstrated a relationship between increased lipid core and higher cerebral events (Ouhlous et al. 2005; Takaya et al. 2006). Similarly using CT, it has been found in severe lesions of >70% stenosis, that there is a positive association between lipidic plaque and symptoms while there is an inverse association between calcified plaque and symptoms (Saba et al. 2009). These studies suggest that plaque
composition may influence the stability of the lesion and contribute to symptoms and clinical events.

Plaque ulceration could be an important feature to identify as it may change management. It has been shown that angiographic plaque surface irregularity was an independent predictor of ipsilateral stroke while on medical treatment at all degrees of stenosis (Rothwell et al. 2000). CT is particularly sensitive at detecting ulceration (up to 94%) compared with ultrasound which only has a sensitivity of 37% (Bluth et al. 1988; Saba, Caddeo, et al. 2007).

However, there are conflicting reports regarding the importance of features such as plaque ulceration and intra-lesion haemorrhage or thrombosis. In a recent large study (n = 673), the presence of intra-lesion thrombus was highly predictive of symptomatic carotid stenosis while extensive calcification was associated with lack of symptoms (Eesa et al. 2010). In contrast, the authors did not find any correlation between plaque hypo-density (presumably ‘lipidic’ plaque) or plaque ulceration in predicting symptoms. However, it is uncertain whether CT can differentiate intra-plaque haemorrhage from necrotic core as they both appear as areas of hypo-density in plaques (Culebras et al. 1988; Oliver et al. 1999).
1.5.3 Magnetic Resonance Imaging

The use of MRI to investigate carotid stenosis has been studied extensively and is perhaps the most capable in providing information on carotid plaque morphology. With regards to stenosis detection, MR angiography using ‘Time of Flight’ (TOF) images have been compared favourably with invasive angiography with sensitivity of 93% and specificity of 88% (Yucel et al. 1999). It provides a strong vascular signal even when the arterial blood flow is slow. The technique takes about 10-15 minutes and movements or swallowing can cause artefacts especially with tight stenosis (Nederkoorn, van der Graaf, et al. 2002).

Contrast enhanced MRA using a paramagnetic agent such as gadolinium offers a significant improvement and has become the technique of choice for MRA. It provides better contrast between lumen and vessel wall, higher quality images and being less prone to artefacts. However, the spatial resolution is still 2-3 times worse than digital subtraction angiography or CTA. Gadolinium is contraindicated in patients with renal failure as the risk of nephrogenic systemic fibrosis (NSF) is up to 8.4% (Kribben et al. 2009).

The ability of MRI to characterize vessel wall and plaque components have been investigated both in-vitro and in-vivo (Shinnar et al. 1999; Toussaint et al. 1996). By using different contrast weighting (TOF, T1, T2, proton density) on the same area, various plaque components can be identified on MRI and validated against histology (Saam et al. 2005; Shinnar et al. 1999). This technique has been used to accurately identify lipid-rich cores and intra-plaque haemorrhage of carotid plaques (Yuan et al. 2001). Researchers from the University of Washington who have published extensively in the area, summarized the interpretation of multi-contrast weighted MRI of carotid plaque morphology in a recent review article (Dong et al. 2009) as outlined in Table 1.5.1.
Table 1.5.1. MRI criteria for plaque tissue components (modified from Dong et al. 2009)

<table>
<thead>
<tr>
<th>Plaque Component</th>
<th>TOF</th>
<th>T1W</th>
<th>T2W</th>
<th>PDW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent IH</td>
<td>+</td>
<td>+</td>
<td>- / +</td>
<td>- / +</td>
</tr>
<tr>
<td>LR-NC</td>
<td>o</td>
<td>o</td>
<td>-</td>
<td>- / o</td>
</tr>
<tr>
<td>Fibrous tissue</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>Intimal calcifications</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

IH denotes intraplaque haemorrhage; LR-NC denotes lipid rich: necrotic core;

“+“ indicates hyperintense compared to reference tissue; “o“ indicates similar intensity;

“-“ indicates hypointense to reference tissue.

The ability to characterize plaque raises the possibility of identifying ‘vulnerable plaques’ which are at risk of complications rather than merely documenting stenosis severity.

Retrospective histology studies of carotid lesions in patients with TIA or stroke have found fibrous cap rupture in 74% of symptomatic patients compared with 32% of asymptomatic patients (Carr et al. 1996). Plaque rupture was also found more commonly in major stroke (67%) compared with TIA (23%) or patients without prior symptoms (13%) (Spagnoli et al. 2004). Furthermore the presence of acute thrombus was associated with plaque rupture in 90% of patients with stroke (Spagnoli et al. 2004).

An important part of ‘vulnerable plaque’ identification is visualization of the fibrous cap. The University of Washington group utilized specialized sequences which later became known as 3-dimensional TOF to distinguish thick, thin and disrupted fibrous cap (Hatsukami et al. 2000). The same group later applied ‘contrast-enhanced’ or CE-MRI
using gadolinium to enhance the fibrous cap thereby making it easier to identify and measure against the adjacent non-enhancing lipid-core (Cai, J et al. 2005; Yuan et al. 2002).

Apart from visualization of the fibrous cap, the other important feature is correctly identifying thrombus which may be the consequence of a high risk plaque. The ability to discriminate between intraplaque and juxtaluminal thrombus using multi-contrast weighted MRI have been shown to be highly accurate (96%) against histology (Kampschulte et al. 2004). The authors found the underlying plaque lesion of intraplaque haemorrhage to be lipid-rich in 55% of cases while juxtaluminal thrombus had an underlying calcified type lesion in 70% of cases.

In order to answer the clinical question of whether carotid plaque characterization by MRI would be predictive of future events, the University of Washington group conducted a prospective study (n=154) with a mean follow up of 38 months (Takaya et al. 2006). They found high-risk features on multi-contrast weighted MRI to be thin or ruptured fibrous caps, intraplaque haemorrhage, larger percentage lipid-rich/necrotic cores and larger maximal wall thickness.

In summary, it is clear from MRI studies of carotid lesions that certain plaque characteristics are associated with higher risk of subsequent events or complications. While it is uncertain the exact incremental value of identifying these features may have over purely stenosis severity, the notion of identifying ‘vulnerable plaques’ for potential future therapies remain very attractive.
1.5.4 Management of Carotid Stenosis

1.5.4.1 Medical management

The management of severe carotid stenosis has traditionally been focused on revascularization but it is important not to neglect medical therapy. Aggressive modification of cardiovascular risk factors including hypertension, diabetes, hyperlipidaemia, smoking, alcohol intake and obesity have been recommended in recent AHA/ASA guidelines (Furie et al. 2010).

The use of anti-platelet agents for secondary prevention of stroke (non-cardioembolic cause) has been approved by regulatory bodies for aspirin, aspirin/dipyridamole combination, clopidogrel and ticlopidine. Selection amongst these agents depends on multiple factors including efficacy, cost, safety and side effects. Aspirin has remained first line therapy based on low cost and good efficacy but a small risk of gastrointestinal bleeding (0.4% per year) when taken in low dose (Johnson et al. 1999). The combination of aspirin with dipyridamole showed a significantly greater reduction in stroke compared to aspirin alone but also commonly caused side-effects of headache and gastrointestinal symptoms (Diener et al. 1996). Although ticlopidine may be more effective than aspirin, concerns over neutropenia and thrombotic thrombocytopenic purpura have rendered it out of favour (Gent et al. 1989; Hass et al. 1989). Clopidogrel has been compared to other anti-platelet agents listed above and even in combination with aspirin, but has not been shown to be superior to any (Furie et al. 2010). In particular, the combination with aspirin is not superior to clopidogrel alone in the MATCH trial (Diener et al. 2004) or aspirin alone in the CHARISMA trial (Bhatt et al. 2006).
1.5.4.2  Carotid Endarterectomy

Amongst the numerous publications demonstrating the benefit of carotid endarterectomy (CEA) over medical therapy for symptomatic severe stenosis (>70%), are three major prospective randomized trials: North American Symptomatic Carotid Endarterectomy Trial (NASCET 1991), European Carotid Surgery Trial (ECST 1991) and Veterans Affairs Cooperative Study Program (VACS) (Mayberg et al. 1991). Analysis of these trials found that CEA carries a 30-day stroke and death rate of 6.2% (absolute risk reduction 15.6%) but it did not reduce stroke risk for those with <50% stenosis (Rothwell et al. 2003).

Surgery for symptomatic moderate carotid stenosis (50-69%) is controversial with a subsequent study of NASCET showing modest benefit at 5 years (stroke rate 15.7% vs 22.2%, p=0.045) over medical therapy (Barnett et al. 1998). Therefore, the recommendation in this group is for CEA only if the perioperative risk has been assessed to be less than 6% (Furie et al. 2010).

There has been debate in the literature on optimal timing of CEA after a TIA or stroke, usually at 2 weeks or after 6 weeks. For mild strokes or TIA, surgery within 2 weeks showed the best outcomes, especially for women whose decline in benefit from CEA over time was more rapid than men (Rothwell et al. 2004). There are no randomized trials for severe disabling strokes but small studies found no differences in morbidity for delaying CEA; in some cases of large infarcts, brain swelling and haemorrhagic transformations, early surgery carries an unacceptably high perioperative risk (Brandl et al. 2001; Chaturvedi et al. 2005).
Surgery for asymptomatic carotid stenosis (60-99%) has been studied in three randomized trials (ACAS 1995; Halliday et al. 2004; Hobson et al. 1993). A Cochrane Review of these studies concluded that there is a small absolute risk reduction of 1% per year and relative risk reduction of 30% over 3 years despite an initial 3% perioperative risk of stroke and death (Chambers & Donnan 2005). A recent publication on the 10 year results of the ACST trial showed lasting benefits of CEA in asymptomatic patients with an absolute risk reduction of 5% of stroke (Halliday et al. 2010).

1.5.4.3 Carotid Artery Stenting

Carotid artery stenting (CAS) has emerged since mid-1990s as a less invasive alternative to CEA (Yadav et al. 1997). The advantages include avoidance of general anaesthesia, no risk of damage to surrounding neck structures, less wound infection, shorter recovery time and better access for lesions which extend into base of skull. However, many trials have failed to show superiority of CAS over CEA but possibly worse outcomes in terms of periprocedural stroke in a recent meta-analysis (Meier et al. 2010).

Even though CAS may be minimally invasive, it is not without complications. There is a risk of distal embolization occurring with wiring of the lesion, predilatation, stenting and postdilatation which has been reduced but not eliminated with the use of embolic protection devices (Kastrup et al. 2006). Up to 68% of patients may have periprocedural bradycardia or hypotension, due to carotid baroreceptor stimulation. Hyperperfusion syndrome is a rare and serious complication after CEA or CAS and is thought to be caused by impaired cerebral autoregulation with changes in cerebral haemodynamics. It occurs in
approximately 1% of CAS patients who usually have very tight stenosis (mean of 95%) and concurrent contralateral stenosis (Abou-Chebl et al. 2004).

Acute and sub-acute stent thrombosis have been reported in 0.5 to 2% of patients and some cases may be related to inadequate or discontinued antiplatelet therapy (Diethrich et al. 1996; Roubin et al. 1996). Beyond 30 days, early restenosis is mainly due to neointimal hyperplasia (Willfort-Ehringer et al. 2003). All CAS procedures use bare metal stents and a review of 34 studies showed a 6% rate of angiographic restenosis (>50%) at 1 year (Groschel et al. 2005). This is better than the rates of early restenosis in 12 to 18 months following CEA which ranged between 5.2 to 11.4% (Frericks et al. 1998; McCabe et al. 2005; Moore et al. 1998). However, more recent techniques of patch closure following CEA have yielded superior results with regards to perioperative strokes and restenosis (AbuRahma et al. 1998). Long term data on CAS restenosis is lacking but one study showed the rate at 10 years to be 6.8%, suggesting the majority of restenosis tends to occur during the initial year (Bergeron et al. 2005). There is no data in the use of drug eluting stents in CAS but a study has reported its use in 5 patients with good results (Gupta et al. 2006).

1.5.4.4 Embolic protection devices (EPD)

A feared complication of CAS is stroke. An early study reported 1% major and 4.8% minor stroke rate at 30 days (Roubin et al. 2001). Systems to guard against distal embolization during CAS have been developed and was first used in 1998 (Henry et al. 1999). After placement of guidewire, EPDs deploy momentarily before dilatation of lesion
or stenting and is retrieved at the end of the procedure (Zahn et al. 2004). Two types of embolic protection devices are now in use to minimize embolic complications of CAS.

1.5.4.4.1 Proximal protection devices

Occlusion balloons are deployed in the external carotid artery and common carotid artery, resulting in retrograde blood flow in the internal carotid artery, which prevents embolization to the brain. This relies to some extent on the collateral blood supply of the circle of Willis causing a back pressure, hence retrograde flow. At the end of the procedure before balloon deflation, the blood in the internal carotid artery is sucked out along with any debris present. The main advantage is that there is minimal disturbance of the lesion and the entire procedure is carried out under embolic protection. Problems with this type of device include larger size (10 Fr introducer sheaths), lost visualisation of stenosis during flow reversal and cerebral ischaemia which may occur (Brown 2004).

1.5.4.4.2 Distal protection devices

These devices occlude or filter distal blood flow to catch the debris dislodged during CAS. Distal filter devices usually consist of a flexible metal skeleton coated by a membrane of polyethylene or net of Nitinol wires that contain holes varying from 80-200 μm (Reimers et al. 2001). Therefore, they must pass across the stenosis which may dislodge the emboli before angioplasty and stenting. Tight lesions may need to be predilated prior to placement of the device and vessel wall injury may be possible (Bates et al. 2007). A commonly used distal device is Angioguard (Cordis, USA) which has a pore size of 100 μm (Yadav et al.)
2004) – Figure 1.5.1. It was used in every CAS patient of the SAPPHIRE trial, where the stroke rate was 5.8% compared to CEA (7.7%) (Yadav et al. 2004).

![Figure 1.5.1. Angioguard distal protection device with umbrella shaped net (Courtesy of Cordis Corporation, Australia).](image)

### 1.5.4.4.3 Effectiveness of EPD

There are no randomized trials comparing CAS with and without EPD. Most prospective stent registries (Brown 2004; Eckert & Zeumer 2003; Reimers et al. 2004; Zahn et al. 2004) and case series (Al-Mubarak et al. 2002; Al-Mubarak et al. 2001; Chan et al. 2005; Cremonesi et al. 2003; Henry et al. 1999; Reimers et al. 2001; Theron et al. 1996; Whitlow et al. 2002) report significantly lowered risk of stroke with the use of EPD at the time of CAS. However randomised controlled trials provide conflicting data about EPD efficacy. A systematic review in 2003 showed that EPD procedures were associated with lower rate of minor stroke (0.5 vs 3.7%) and major stroke (0.3 vs 1.1%) compared to those procedures without an EPD (Kastrup et al. 2003). The EVA-3S and SPACE trials have suggested that EPD are not effective in prevention of stroke, although these trials were limited by data from small subgroup analysis (Mas et al. 2006; Ringleb et al. 2006).
1.5.4.4 Complications of EPDs

During CAS using filter type embolic protection device, there can be an angiographic appearance of significant reduction in antegrade flow in the ICA proximal to the filter device which is termed "slow flow phenomenon" (Angelini et al. 2002; Reimers et al. 2004). The underlying pathogenesis is uncertain. A hypothesis is that microemboli and debris containing plaque elements may result in blockage of the filter membrane pores, impeding antegrade flow, and therefore causing a stagnant column of blood containing debris in the artery proximal to the filter. One single-centre study of 453 CAS using filter type EPDs found slow flow phenomenon in 9% of procedures (Casserly et al. 2005). Among patients with slow flow, the 30-day incidence of stroke and death was 9.5% compared to 2.9% in patients with normal flow. Significant multivariate predictors of the event include a recent history of TIA/stroke, increased patient age, and increased stent diameter.

Other complications such as haemodynamic intolerance of balloon occlusion or congested filters, arterial spasm and dissection and device retrieval difficulties are also reported. (Cremonesi et al. 2003; Eckert & Zeumer 2003; Reimers et al. 2001)
1.5.4.5 Randomized Trials – CAS v CEA

There have been a number of trials comparing CAS to CEA, often using composite endpoints of stroke and death at various time points. Early trials like CAVATAS reported comparable 30-day stroke or death (6%) in both groups but the surgical outcome was worse than those reported in major CEA trials (CAVATAS 2001). The SAPPHIRE trial where a distal protection device (Angioguard, Cordis, USA) was used in CAS patients showed non-inferiority to CEA (Yadav et al. 2004). At 1 year, there was an absolute reduction of 7.9% in composite endpoint (stroke, AMI, death) for CAS. However, this was largely due to the higher incidence of AMI in the CEA group.

More recent and larger trials like EVA-3S (Mas et al. 2006) and SPACE (Ringleb et al. 2006) were both stopped prematurely due to safety and futility in reaching non-inferiority as they recorded higher 30-day stroke and death endpoints for CAS. Criticisms of these trials included operator inexperience and low use of distal protection devices.

A recent trial (ICSS) of 1713 patients showed much worse outcomes for CAS (Ederle et al. 2010). CAS had worse rates of stroke, all-cause mortality and the primary composite endpoint (stroke, death or procedural AMI) at 120 days (8.5% v 5.2%; p=0.006).

One of the largest and most highly anticipated trials (CREST) was published recently showing a significantly higher stroke rate in CAS group at 30 days. This occurred despite a lower stroke and death rate in comparison to other trials, reflecting the general high experience of the interventionalist. In contrast there was a higher peri-procedural AMI rate
in the CEA group. However the composite endpoint (stroke, AMI, death, subsequent ipsilateral stroke) was not different between the two groups during the 4 year follow-up period. The investigators also found that regardless of symptoms, CAS has greater efficacy in patients <70 years old whereas CEA was superior for patients > 70 years old.

A meta-analysis with included 11 trials, but not CREST, concluded that CEA was superior to CAS for short term outcomes but not significantly different for intermediate term outcomes (Meier et al. 2010). While the risk of peri-procedural AMI was higher in the CEA group, it had a lower risk of strokes and death compared to CAS. This finding was similar to CREST where post hoc analyses suggests that stroke had a greater impact on overall health compared to peri-procedural AMI (Davis & Donnan 2010).

Therefore, current guidelines recommend that CEA be the initial treatment choice for severe carotid stenosis (Furie et al. 2010). CAS is an alternative for symptomatic patients where medical conditions exists which would greatly increase the risk for surgery, in the setting of difficult surgical access or restenosis after CEA.
1.5.5 Conclusions

Imaging plays an important role in both primary and secondary prevention of stroke due to carotid artery stenosis. Whilst stenosis severity remains the main trigger for revascularization, imaging of the vessel wall and plaque have been promising in trying to further risk stratify patients who have “vulnerable plaque”. Multi-contrast MRI has emerged as an imaging technique that is able to demonstrate various carotid plaque types and has been validated against histology.

Carotid artery stenting appears to be an attractive alternative to carotid endarterectomy due to its minimally invasive nature. However, recent randomized trials have demonstrated a higher short-term rate of stroke and death, which is primarily driven by minor strokes, when compared with carotid endarterectomy. The risk of stroke during carotid artery stenting has been reduced but not eliminated by the use of embolic protection devices.
Chapter 2

METHODS
2.1 Saphenous Vein Graft Intervention

The study included 27 consecutive subjects with anginal symptoms in whom conventional coronary angiography revealed a significant lesion in a saphenous vein bypass graft suitable for PCI. The study protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and all subjects gave written informed consent. All subjects underwent computed tomography angiography by a 16-detector row MDCT and MRI before SVG PCI. At the time of SVG PCI, a distal protection device (FilterWire EZ, Boston Scientific USA) was deployed and bare metal stents were used on all lesions. Unfractionated heparin was given as a bolus intravenously on table prior to intervention at a dose of 100 IU/kg. All patients were on aspirin and clopidogrel. This study excluded subjects presenting with an acute ST elevation myocardial infarction, severe left ventricular impairment (EF < 25%), lesions within 2.5 cm of distal anastomosis, lesions < 50% stenosis on angiography and inter-current illness or infection which may elicit a systemic inflammatory response.

2.1.1 Multi-detector computed tomography

All patients underwent CT angiography prior to SVG PCI utilising a 16-detector row CT (Sensation 16, Siemens Medical Solutions, Germany). Patients were in sinus rhythm but did not receive additional beta-blockade prior to scan. In comparison to native coronaries, SVGs are larger and relatively immobile. MDCT data were acquired using 12 x 0.75 mm collimation, 420 ms gantry rotation, 2.8 mm table feed per rotation, 400 mAs with ECG modulation, and tube voltage of 120 kV (Ropers et al. 2003). A bolus of 100 mL of contrast agent (Isovue 370, Iopamidol 52.8 g/70 mL, Bracco) was injected intravenously.
Transaxial images (slice thickness 1.0 mm, increment 0.5 mm) were reconstructed using an ECG-gated half-scan reconstruction algorithm giving a temporal resolution of 210 ms. The CT data set of axial and multi-planar reformatted images were analysed by two independent investigators using the Leonardo (Siemens Medical Solutions, Germany) and Image Pro Plus (Media Cybernetics, USA) software packages, blinded to the results of the intervention and embolic material weight. Cross sections of the lesion perpendicular to the lumen were obtained at 1 mm intervals automatically utilizing Syngo Circulation & Vessel View software (Siemens Medical Solutions, Germany). The display setting used for lumen and plaque quantification followed previously published protocols (Leber et al. 2005). To obtain the window setting for lumen measurements, the width is reduced to 1 Hounsfield Unit (HU) and the level is set to 65% of the mean intensity measured in the lumen. In determining the outer vessel boundaries, the window width was set to 155% of the mean value within the lumen and the level at 65% of the mean value. After application of the appropriate window settings, the lumen and vessel boundaries were manually traced utilizing the Image Pro Plus software. The plaque volume was the result of the sum of these cross sectional areas.

Identification of coronary artery plaque and its calcific or non-calcific nature have been made utilizing existing published definitions (Achenbach et al. 2004; Leber et al. 2005). On every cross-sectional CT image, a region of interest (ROI) was placed over the atheroma. The ROI was the largest circle that could fit into the atheromatous area on the image without involving the lumen or outer vessel wall. The size of the ROI area (mm²) and mean HU value was recorded from each ROI. The entire lesion HU was the mean of all the HU from the cross sectional images. A plaque with HU less than 60 was defined as lipidic, between 60 and 130 HU as fibrotic and above 130 to be calcific. These empiric
definitions were based upon published data using the identical MDCT imaging system (Leber et al. 2004).

### 2.1.2 Magnetic Resonance Imaging

Patient underwent cardiac MRI utilizing a commercial 1.5 Tesla MR system (Siemens Sonata, Siemens Medical, Germany). Patients with contraindications to MRI in general (eg. permanent pacemaker, ferromagnetic metallic implants, claustrophobia) were excluded. Standard initial fast scout gradient-echo images (transverse, coronal, sagittal) were used to locate the course of the SVGs. Black-blood turbo spin echo images (T1 weighted) using ECG gating were obtained over multiple breath-holds (10-15s) for plaque analysis as per published literature (Fayad et al. 2000). These images were orthogonal cross-sections of the SVGs along the length of the lesion. The imaging parameters were: TR = 2 RR intervals (>800ms) / TE 7 ms, slice thickness of 5mm and no-gaps in between slices. The field of view was 18 cm with a 384 x 384 matrix yielding an in-plane resolution of 0.47 mm.

Images were analysed by two independent investigators using the Leonardo (Siemens Medical Solutions, Germany) and Image Pro Plus v5.1 (Media Cybernetics, USA) software packages, blinded to the results of the intervention and embolic material weight. The lumen and outer vessel wall were traced to obtain the cross-sectional area of plaque. The volume was derived from multiplying this area by 5 as the slice thickness was 5mm.
2.1.3 Intravascular Ultrasound

Patients underwent IVUS imaging at the time of their PCI, just prior to any angioplasty or stenting has occurred. A standard 0.014” angioplasty guide wire was inserted pass the lesion and 200mcg of glyceryl trinitrate (GTN) was administered down the SVG. A 3.3 Fr 40mHz Atlantis SR Pro IVUS catheter (Boston Scientific, Natick, Massachusetts) was used to obtain images under continuous automatic pullback speed of 0.5mm /s to the aorto-graft ostium (Clearview IVUS system, Boston Scientific, Natick, Massachusetts). The images were stored digitally on CD and transferred for offline analysis using dedicated software (EchoPlaque, Indec Systems Inc, USA). Plaque volume was derived utilizing selected IVUS frames exactly 1 mm apart for analysis to derive each plaque area then summated for the lesion length as per previous publications and guidelines (Hong, Mintz, et al. 1999; Mintz et al. 2001).

2.1.4 Distal Protection Device.

Patients had the FilterWire EZ (Boston Scientific, USA) deployed during SVG PCI. In order to obtain the net embolic material weight, each FilterWire was processed by the same senior laboratory technician who was blinded to all the data in this study. Specimens were received in individual containers filled with formalin. All fluid was drained and specimen left to stand for the same defined period of time before the FilterWire containing embolic material was weighed intact on a calibrated scale (August Sauter GmbH D-7470, Albstadt 1-Ebingen, Germany). The FilterWire was dissected and all embolic material was removed with a probe and flushed clean. The empty FilterWire was then blotted dry to remove any remaining fluid before re-weighed on the same scale.
2.1.5 **Histopathology.**

Once the material had been removed from the FilterWires, it was spun down using a centrifuge (Heraeus Digifuge GL, Hanau, Germany). In order to fix the lipid component, osmium tetroxide was applied and subsequently irrigated to remove excess osmium. The material was then prepared in 15% bovine serum albumin and allowed to set before embedding into paraffin blocks. These blocks were bisected vertically and stained for light microscopy with haematoxylin & eosin, Picro Mallory (fibrin), Verhoeff-van Gieson (elastin & collagen), and oil red ‘o’ (lipid). High resolution 12 megapixel TIFF images of slides were obtained using a microscope and dedicated camera (Olympus BX41 and DP70, Olympus Optical Co, Japan). These images were then analysed by an expert pathologist and cardiologist utilizing semi-automated imaging software (Image-Pro Plus v5.1, Media Cybernetics, USA) to identify plaque components: thrombus, fibrous, cholesterol clefts, lipid and red blood cells. The absolute and percentage area of each component were obtained.

2.1.6 **Statistical evaluation.**

All analyses were performed with SPSS 19.0 for Windows (IBM SPSS Statistics, USA). Data is represented as mean ± standard deviation or median and interquartile range as appropriate. Spearman’s rank correlation and Mann-Whitney tests were used for non-parametric data and unpaired t-tests used where appropriate. All p values are two-tailed with p<0.05 indicating statistical significance. The inter-observer variability of MDCT measurements was determined by coefficient of variance and Bland-Altman plots.
2.2 Carotid Artery Stenting

Patients were referred for carotid stenting based on clinical symptoms consistent with a previous transient ischaemic attack or stroke and previous imaging (Doppler ultrasound or CT) which revealed a stenosis >70% of the corresponding carotid artery. The study protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and all subjects gave written informed consent. These individuals underwent MRI scanning of the carotid artery before undergoing stenting. All patients had distal protection devices (Angioguard, Cordis, USA) placed just prior to stenting. Unfractionated heparin was given as a bolus intravenously on table prior to intervention at a dose of 100 IU/kg. All patients were on aspirin and clopidogrel. Patients were excluded if they had contraindications to MRI scanning, renal failure, allergy to contrast, or unable to give informed consent.

2.2.1 Magnetic resonance imaging

Patients underwent MRI of the common and internal carotid arteries using a dedicated phased-array carotid coil on a 1.5T MRI system (Siemens Sonata, Siemens Medical, Germany). Multi-contrast images were obtained (TOF, T1W, PDW and T2W) at the common carotid just below the bifurcation and 6 consecutive images of 3mm slice thickness (no gaps) starting at the bifurcation, covering the internal carotid artery. Parameters for the sequences were (i) 2D-TOF (TR/TE 28/6.5 ms, flip angle 50) (ii) T1W black blood turbo spin echo (TR/TE 900/7.7 ms) (iii) PDW turbo spin echo (TR 3RR, TE
(iv) T2W turbo spin echo (TR 3RR, TE 69 ms). All images were cardiac gated, field of view [FOV] 12 cm, thickness 3mm, 256 x 256 matrix, number of excitations 2. Voxel size was 0.46 x 0.46 x 3.0 mm.

Images were transferred for offline analysis using specialized software - Image Pro Plus v5.1 (Media Cybernetics, USA). The luminal and vessel wall areas were traced manually and the plaque area was derived from the difference of the two. The plaque volume was the summation of the plaque areas multiplied by the slice thickness.

Tissue characterization of carotid plaque was determined by measuring the signal intensity relative to the adjacent sternocleidomastoid muscle as described in Table 2.1 below (Dong et al. 2009):

**Table 2.1. MRI criteria for plaque tissue components (modified from Dong et al. 2009)**

<table>
<thead>
<tr>
<th>Plaque Component</th>
<th>TOF</th>
<th>T1W</th>
<th>T2W</th>
<th>PDW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent IH</td>
<td>+</td>
<td>+</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>LR-NC</td>
<td>o</td>
<td>o</td>
<td>-</td>
<td>- / o</td>
</tr>
<tr>
<td>Fibrous tissue</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>Intimal calcifications</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

IH denotes intraplaque haemorrhage; LR-NC denotes lipid rich: necrotic core;

“+” indicates hyperintense compared to reference tissue; “o” indicates similar intensity;

“−” indicates hypointense to reference tissue.
2.2.2 Histopathology

Embolic debris captured by the Angioguard device during carotid stenting were prepared for analysis. Histopathology and statistical analysis were carried out in the same manner as described for SVG debris.
Chapter 3

ANGIOGRAPHIC PREDICTORS OF IMPAIRED FLOW
DURING SAPHENOUS VEIN GRAFT INTERVENTION
Saphenous vein graft intervention carries a higher morbidity and mortality compared to native coronary interventions largely due to higher risk of myocardial infarction (de Feyter et al. 1993; Holmes et al. 1995; Hong, Mehran, et al. 1999). Increased tendency of atheromatous plaque embolism to the microcirculation has been implicated, sometimes resulting in the ‘slow flow’ phenomenon (Baim et al. 2002; Stone et al. 2003). Despite the introduction of distal protection devices, this risk has been reduced but not eliminated.

‘Slow flow’ has traditionally been defined as angiographic flow of epicardial vessels visually graded to be less than TIMI 3. The TIMI flow grading system was originally developed to determine reperfusion after thrombolysis and identifies patients with higher risk for adverse events ('The Thrombolysis in Myocardial Infarction (TIMI) trial. Phase I findings. TIMI Study Group' 1985). However, this system is categorical, subjective and may be open to inter-observer variability, especially with regards to grading TIMI 2 flow (Gibson et al. 1999).

The TIMI frame count method of quantifying flow is arguably more accurate but requires the calculation of cineframes required for dye to travel from the ostium to a distal landmark in a vessel. In native coronaries it is an independent predictor of in-hospital mortality and can identify at risk sub-groups within TIMI 3 flow (Gibson et al. 1999).

We sought to investigate the relationship between the amount of embolic material caught in a distal protection device and reduced flow as determined by TFC post SVG intervention.
3.1 Methods

This study was a prospective observational study involving consecutive patients referred for saphenous vein graft intervention at a single centre. The study protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and all subjects gave written informed consent. The study enrolled 27 consecutive subjects with anginal symptoms in whom conventional coronary angiography revealed a significant lesion in a saphenous vein bypass graft suitable for PCI. Three patients were excluded due to inadequate angiogram cineframes for analysis, leaving a final number of 24 patients. At the time of SVG PCI, a distal protection device (FilterWire EZ, Boston Scientific USA) was deployed and all patients were on aspirin and clopidogrel. Unfractionated heparin was given as a bolus intravenously on table prior to intervention at a dose of 100 IU/kg. Bare metal stents were used on all lesions. This study excluded subjects presenting with an acute STEMI, severe left ventricular impairment (EF < 25%), lesions within 2.5 cm of distal anastomosis, lesions < 50% stenosis on angiography and inter-current illness or infection which may elicit a systemic inflammatory response.

In order to obtain the net embolic material weight, each FilterWire was processed by the same senior laboratory technician who was blinded to all the data in this study. Specimens were received in individual containers filled with formalin. All fluid was drained and specimen left to stand for the same defined period of time. Then the FilterWire containing embolic material was weighed intact on a calibrated scale (August Sauter GmbH D-7470, Albstadt 1-Ebingen, Germany). The FilterWire was dissected and all embolic material was
removed with a probe and flushed clean. The empty FilterWire was then blotted dry to remove any remaining fluid before re-weighed on the same scale.

Quantitative coronary analysis (QCA) was performed on angiograms during intervention using the software QCA-CMS 6.0 (Medis Medical Imaging Systems, Netherlands). Two cardiologists blinded to the results determined the TIMI frame counts of each case in accordance with published literature (Al-Mousa et al. 1998; Gibson et al. 1996). We recorded the number of cine frames required for dye to travel from the ostium of the graft to the graft anastomotic site before intervention, after stent deployment with FilterWire still insitu, and at the end of procedure (Figure 3.1). We divided the patients into two groups based on their TFC at the end of the intervention being better or worse than at the start of the case. The latter group must have a deterioration in TFC of three frames.

Estimated plaque volume of the lesion was derived by assuming the vessel is a uniform cylinder as previously described (Giugliano et al. 2005). This is done from simple mathematical formula: \[ \pi \left((\text{lesion length})[(\text{RVD}/2)^2-(\text{MLD}/2)^2]\right) \] where RVD denotes reference vessel diameter and MLD, minimal lumen diameter. The SVG degeneration score is the percentage of the vessel length which has at least 20% stenosis. The score is out of 4 corresponding to the quartiles of the diseased vessel length (Giugliano et al. 2005).

All analyses were performed with SPSS 19.0 for Windows (IBM SPSS Statistics, USA). Data is represented as mean ± standard deviation or median and interquartile range as appropriate. The primary endpoint was embolic material weight from the FilterWires. Spearman’s rank correlation and Mann-Whitney tests were used for non-parametric data and unpaired t-tests used where appropriate. All p values are two-tailed with p<0.05.
indicating statistical significance. The inter-observer variability of TFC measurements was determined by coefficient of variance.

**Figure 3.1.** Angiograms of same patient before PCI (panels A, B, C) and after PCI (panels D, E, F). In panel C, the TIMI frame count (TFC) was 25 when the contrast reached the distal graft anastomosis. In panel F, at the same TFC of 25, the contrast has only progressed to mid graft, just proximal to where a stent was deployed to treat a long segment of stenosis. This patient had no-reflow.
3.2 Results

A total of 26 lesions from 24 patients were analysed. Every FilterWire yielded some material, with the median weight of 8mg (IQR 3.75, 13.3). The inter-observer variability in determining the TFC was excellent with a co-efficient of variance at 2.3%. At the time of SVG intervention, two patients suffered AMI from no-reflow (TIMI flow 0-1) and one patient died from AMI a few days post intervention. Apart from the two patients who suffered myocardial infarctions during intervention, all other patients achieved TIMI 3 flow at the end of their case.

Table 3.1. Baseline patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.3 ± 8.6</td>
</tr>
<tr>
<td>Male</td>
<td>22 (95.7)</td>
</tr>
<tr>
<td>Age of SVGs (years)</td>
<td>14.3 ± 4.3</td>
</tr>
<tr>
<td>ACS presentation</td>
<td>11 (44)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>10 (43.5)</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>3 (13.1)</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>13 (56.5)</td>
</tr>
<tr>
<td>Statins</td>
<td>22 (95.7)</td>
</tr>
</tbody>
</table>

The baseline patient demographic and angiographic data are summarized in Tables 3.1 & 3.2. The patients were predominantly male with long lesions in old vein grafts.
Table 3.2. Angiographic characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion length (mm)</td>
<td>18.5 (11.8, 26.9)</td>
</tr>
<tr>
<td>Total stent length (mm)</td>
<td>20.5 (18, 29)</td>
</tr>
<tr>
<td>Number of stents (mean)</td>
<td>1.15 (±0.5)</td>
</tr>
<tr>
<td>Reference vessel diameter (mm)</td>
<td>4.42 (±1.22)</td>
</tr>
<tr>
<td>Minimal luminal diameter (mm)</td>
<td>1.38 (±0.83)</td>
</tr>
<tr>
<td>New thrombus formation</td>
<td>7 (26.9%)</td>
</tr>
<tr>
<td>Eccentricity</td>
<td>13 (50%)</td>
</tr>
<tr>
<td>Ulceration</td>
<td>8 (30.8%)</td>
</tr>
<tr>
<td>Calcification</td>
<td>4 (15.4%)</td>
</tr>
<tr>
<td>Angulation (&gt;45°)</td>
<td>3 (11.5%)</td>
</tr>
<tr>
<td>Target Vessel</td>
<td></td>
</tr>
<tr>
<td>SVG to LAD</td>
<td>22.2%</td>
</tr>
<tr>
<td>SVG to LCX</td>
<td>29.6%</td>
</tr>
<tr>
<td>SVG to RCA</td>
<td>48.2%</td>
</tr>
<tr>
<td>SVG degeneration score (%)</td>
<td>25.8 (±16.0)</td>
</tr>
<tr>
<td>TIMI flow pre</td>
<td></td>
</tr>
<tr>
<td>0/1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>11.5%</td>
</tr>
<tr>
<td>3</td>
<td>88.5%</td>
</tr>
<tr>
<td>Estimated plaque volume (mm³)</td>
<td>245.7 (148, 374)</td>
</tr>
</tbody>
</table>

LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery
There were 7 lesions where the TFC was worse post PCI (median TFC change: +16, IQR 5.5 to 47). In contrast, 19 lesions had improvement in TFC post PCI (median TFC change: -4, (IQR -10 to -1). Figure 3.2 shows a significantly larger amount of embolic material caught by the FilterWire in the group where the TFC was worse at the end of the case (15.1 mg v 6 mg; p=0.009). The peak creatinine kinase post intervention was significantly higher in this group (129 IU/L, IQR 90-737) compared with those with improved TFC (79 IU/L, IQR 52-106; p = 0.046).

![Emboli & TFC post PCI](image)

**Figure 3.2** Box and whisker plots showing patients with worse TIMI frame count (n=7) after PCI had significantly greater embolic material weight compared to patients who had improved TIMI frame count (n=19).

Angiographic new thrombus formation was observed in seven cases which prompted the interventionalist to commence abciximab during the case. This was associated with worse TFC at end of procedure (p=0.006) and when greater than one stent was used (p=0.017), as determined by Fisher’s exact test. Not unexpectedly this was also found in relation to longer lesion lengths (29.2 v 17.2 mm; p=0.035).
Table 3.3 display the results of any correlations of common angiographic parameters to the amount of embolic material caught by the FilterWire.

<table>
<thead>
<tr>
<th>Continuous Variables</th>
<th>rs value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion length</td>
<td>0.369</td>
<td>0.063</td>
</tr>
<tr>
<td>Total stent length</td>
<td>0.344</td>
<td>0.085</td>
</tr>
<tr>
<td>Number of stents used</td>
<td>0.463</td>
<td>0.017</td>
</tr>
<tr>
<td>Reference vessel diameter</td>
<td>0.483</td>
<td>0.012</td>
</tr>
<tr>
<td>Estimated plaque volume</td>
<td>0.533</td>
<td>0.005</td>
</tr>
<tr>
<td>CK peak</td>
<td>0.130</td>
<td>0.537</td>
</tr>
<tr>
<td>SVG degeneration score</td>
<td>0.199</td>
<td>0.329</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Categorical variables</th>
<th>Present</th>
<th>Absent</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eccentric lesion</td>
<td>7.0 mg</td>
<td>10.0 mg</td>
<td>Ns</td>
</tr>
<tr>
<td>Ulceration</td>
<td>6.0 mg</td>
<td>8.0 mg</td>
<td>Ns</td>
</tr>
<tr>
<td>Calcification</td>
<td>7.0 mg</td>
<td>8.0 mg</td>
<td>Ns</td>
</tr>
<tr>
<td>Lesion angulation &gt; 45°</td>
<td>6.0 mg</td>
<td>8.0 mg</td>
<td>Ns</td>
</tr>
<tr>
<td>Balloon post-dilatation</td>
<td>8.0 mg</td>
<td>5.9 mg</td>
<td>Ns</td>
</tr>
<tr>
<td>New thrombus formation</td>
<td>15.1 mg</td>
<td>8.0 mg</td>
<td>0.072</td>
</tr>
</tbody>
</table>

Spearman correlation used for continuous variables and Mann Whitney for categorical variables. CK denotes creatinine kinase; SVG denotes saphenous vein graft.
3.3 Discussion

The early distal protection devices trials demonstrated a reduction in risk of morbidity in SVG intervention but not an elimination of that risk (Baim et al. 2002; Stone et al. 2003). Imperfect apposition of the device against vessel wall, overflow of embolic material in the device and micro-particles smaller than the pore size of the device have been theorized in previous publications (Shaia & Heuser 2005; Stone 2005).

We have demonstrated that despite the use of a distal protection device, there is a relationship between the amounts of embolic material produced and impaired flow as determined by a worse TFC at the end of the case. Of these cases, five out of seven showed significant deterioration of TFC despite a final TIMI 3 flow, demonstrating the finer grading system of TFC in relation to flow. It has been shown in native coronaries that TFC is able to identify subgroups within those with TIMI 3 flow at higher risk as well as being an independent predictor of in-hospital mortality post AMI (Gibson et al. 1999). However, it is unclear if there are any significant clinical sequelae in our group with worse TFC post PCI.

There have been attempts to identify angiographic markers of increased risk during SVG intervention with varying degrees of success. A recent study using FilterWire in SVG PCI, did not find any correlation between the uniplanar area of the FilterWire which was covered with embolic material, and a multitude of angiographic, procedural and clinical variables (van Gaal et al. 2007). In contrast, by using embolic material weight as the endpoint, we were able to better define the degree of plaque embolization. There were
significant correlations between reference vessel diameter, number of stents used and estimated plaque volume from QCA and the amount of embolic debris trapped by our FilterWires.

A larger study of 194 patients undergoing a mixture of coronary and vein graft PCI utilizing FilterWire devices, concluded that longer stent lengths and final TIMI flow < 3 were predictors of ‘visually significant’ debris greater than 1 mm (El-Jack et al. 2007). A recent large study of nearly 4000 patients derived from pooled data from five randomized trials and one registry found two angiographic markers to predict 30-day MACE (Coolong et al. 2008). These two novel markers measured by quantitative coronary angiography were estimated plaque volume (derived from a mathematical formula which treats the vessel as a uniform cylinder) and SVG degeneration score. Our data echo the findings of these two studies where we found positive correlations between debris burden and worse TFC along with increase estimated plaque volume on QCA.

The formation of new thrombus during intervention has been found to carry increased risk of peri-procedural death and myocardial infarction (Boersma et al. 2002). In our cohort, none were on glycoprotein IIb/IIIa at the commencement of the case and abciximab was employed when new thrombus formation was visualized during intervention. We found this to be associated with usage of more than one stent and a worse TFC at the end of the procedure. This finding suggest that prothrombotic factors may have a role resulting in reduced flow post SVG intervention despite the use of distal protection devices which supposedly remove most of the associated particles.
3.3.1 Limitations

This is a small study, and as such we do not have the ability to assess the independence of the factors associated with embolic material weight with multivariate logistic regression. There were only 2 cases of no-reflow which is insufficient for any robust analysis of its predictors. Unfortunately, insufficient data from the cineangiograms of some patients precluded analysis of TIMI myocardial perfusion grade (TMPG) in our cohort. Impaired myocardial perfusion as assessed by TMPG has been shown to have a higher risk of mortality independent of epicardial blood flow in the post thrombolysis setting (Gibson et al. 2000). We did not record the number or pressure of post dilatation balloon inflations which was at the discretion of the operator.

3.4 Conclusion

Our study has demonstrated that despite use of distal protection device, greater embolic material is associated with an increased likelihood of post-procedural flow impairment. Surrogate markers of plaque burden such as reference vessel diameter, greater number of stents used and estimated plaque volume are significant correlates of increasing amount of embolic debris.
Chapter 4

ACCURACY OF MDCT AND MRI COMPARED TO IVUS IN PLAQUE QUANTIFICATION
Intravascular ultrasound (IVUS) is perhaps the best tool available to quantify plaque in the coronary arteries. Its robustness has led to its’ use in a number of atheroma progression trials of the human coronaries (Nicholls et al. 2010). However, this is an invasive technique which carries a small risk of complications. The ability to successfully and accurately measure plaque volumes would be advantageous for documentation of one’s burden of disease and perhaps as a marker for treatment success of future therapies.

While there are a number of plaque quantification studies of the coronaries comparing multidetector computed tomography (MDCT) with IVUS, there are no similar studies for saphenous vein graft (SVG) plaque (Achenbach et al. 2004; Leber et al. 2006). There has been one study investigating the luminal dimensions on MDCT compared with IVUS (Pregowski et al. 2011). In the area of SVG intervention, surrogate markers of plaque burden such as lesion length, SVG degeneration score and estimated plaque volume from quantitative coronary angiography (QCA) has been associated with increased adverse events at 30 days (Coolong et al. 2008; Kirtane et al. 2008).

We sought to investigate the ability of MDCT and MRI to measure SVG lumen, vessel wall and plaque areas as compared with IVUS. From these parameters it would enable us to also calculate the plaque volume.
4.1 Methods

A total of 18 patients with 19 lesions underwent MRI scanning successfully using black blood turbo spin echo sequence as outlined in Chapter 2. MRI image acquisition involves aligning the axis of imaging along the course of the SVG, in order to obtain orthogonal cross sectional images. Each slice of 5mm thickness is obtained during breath holds. Longer SVG lesions would require multiple successive imaging sequences along the path of sometimes tortuous vessels.

In addition, they all underwent MDCT before IVUS as outlined in Chapter 2. Images from all three modalities were matched using anatomical, vessel and lesion landmarks (Figure 4.1). MDCT and IVUS images were analysed every 1mm along the entire length of the lesion while MRI was analysed every 5mm due to the slice thickness of the MRI image. Lumen and vessel wall areas were traced manually using imaging software as described in Chapter 2. MDCT images were threshold accordingly as described by previous published methods (Leber et al. 2005). IVUS images were analysed every 30th frame which corresponded to 1mm apart. In the event that particular frame displayed an artefact, the adjacent frame was used instead. Two independent observers analysed the CT and MRI images. Statistical analysis was conducted as outlined in Chapter 2. Simple correlation and Bland-Altman plots were used to compare dimensions measured by the different modalities.
Figure 4.1. **A.** Coronary angiogram of long SVG lesion. **B.** Corresponding multi-planar reformat CT image with red line indicating location of cross-sectional views for panels D & E. **C.** Corresponding IVUS image of the same SVG lesion showing lumen and outer vessel wall. **D.** Black blood MRI cross section through bulky SVG plaque; magnified inset. **E.** Corresponding CT cross section image; magnified inset.
4.2 Results

Both IVUS and CT were successful in obtaining images along the entire length of the lesions. However, 35.5% of IVUS images were hampered by calcific arcs > 90° and in 23.6% of images, parts of the EEM were beyond the field of view. MRI was only able to image an average of 70.1% of the lesion length due to vessel tortuosity, motion and limitations in breath holds. In order to accurately compare plaque volumes between the three imaging modalities, only the corresponding matched images from IVUS and CT were used in comparison to MRI.

The mean lesion length was $19.2 \pm 8.9$mm. The inter-observer variability (coefficient of variance) for plaque volume was better for CT (3.6%) than MRI (9.9%). Table 4.1 display the vessel measurements along with results of paired t-test of IVUS-CT and IVUS-MRI pairings. There were no differences in lumen area measured by CT or MRI compared to IVUS. However, the vessel area was much larger by CT and almost doubled as measured by MRI. This resulted in large differences in corresponding plaque areas and volumes.

| Table 4.1. CT & MRI vessel measurements compared to IVUS |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | IVUS mean       | CT mean         | p value         | MRI mean        | p value         |
| Lumen area (mm²)| 9.4 ± 3.3       | 8.8 ± 4.1       | 0.217           | 9.2 ± 4.1       | 0.761           |
| Vessel area (mm²)| 23.6 ± 4.9      | 31.3 ± 10.2     | < 0.001         | 43.6 ± 14.9     | < 0.001         |
| Plaque area (mm²)| 14.2 ± 3.0      | 22.5 ± 8.0      | < 0.001         | 34.4 ± 12.1     | < 0.001         |
| Plaque volume (mm³)| 281.9 ± 164.1  | 468.8 ± 358.2   | 0.003           | 719.4 ± 550.0   | < 0.001         |
Results expressed as mean ± standard deviation; p value of paired t-test against IVUS.

Table 4.2 summarizes the correlations for quantification of lumen area, vessel area and plaque volume by CT and MRI as compared to IVUS.

**Table 4.2.** Correlations between CT, MRI with IVUS.

<table>
<thead>
<tr>
<th></th>
<th>CT</th>
<th>MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
</tr>
<tr>
<td>Lumen area</td>
<td>0.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vessel area</td>
<td>0.77</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plaque volume</td>
<td>0.83</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Figures 4.2, 4.3 and 4.4 show the correlations between IVUS & CT in measuring lumen area, vessel wall area and plaque volume respectively. The Bland-Altman plots show that CT was excellent in measuring the lumen area and small over-estimation of the vessel wall area. However, the summation of these small over-estimations resulted in a moderately larger plaque volume which has a pattern of proportional error with increasing volumes.

![Figure 4.2](image-url) **Figure 4.2** Linear regression and Bland-Altman plots of lumen area measured by CT compared to IVUS.
Figure 4.3. Linear regression and Bland-Altman plots of vessel wall area measured by CT compared to IVUS.

Figure 4.4. Linear regression and Bland-Altman plots of plaque volume measured by CT compared to IVUS.

Similarly, figures 4.5, 4.6 and 4.7 show the correlations between IVUS & MRI in measuring lumen area, vessel wall area and plaque volume respectively. The Bland-Altman plots show good agreement between MRI and IVUS in measuring lumen area. However, MRI had significant positive biases in measuring the vessel wall area (mean: +20 mm²) and plaque volumes (mean: +437mm³), with proportional errors as vessel and plaques increased size.
Figure 4.5. Linear regression and Bland-Altman plots of lumen area measured by MRI compared to IVUS.

Figure 4.6. Linear regression and Bland-Altman plots of vessel area measured by MRI compared to IVUS.

Figure 4.7. Linear regression and Bland-Altman plots of plaque volume measured by MRI compared to IVUS.
4.3 DISCUSSION

Previous research has shown the ability to assess SVG patency by cine MRI (Aurigemma et al. 1989), 4-detector CT (Ropers et al. 2001), 16-detector CT (Anders et al. 2006; Martuscelli et al. 2004) and 64-detector CT (Ropers et al. 2006). In assessing graft patency, MRI had a sensitivity of 88% and specificity of 100% (Aurigemma et al. 1989) while 16-detector CT achieved 100% sensitivity and 98% specificity (Anders et al. 2006). However, they often assessed patency as a binary fashion and did not quantify lumen or vessel wall dimensions. Our study is amongst the first to investigate the ability of MDCT and MRI to quantify plaque in SVGs in a direct comparison to IVUS.

In recent years, there have been a number of studies in the ability of MDCT to quantify coronary artery stenosis and to a lesser extent plaque quantification in comparison to IVUS. These studies, utilizing 16-detector (Achenbach et al. 2004), 64-detector (Leber et al. 2006) and dual-source CT (Schepis et al. 2010), found good to excellent correlations of plaque volumes between IVUS and MDCT but only moderate agreement on Bland-Altman plots. MDCT underestimated the plaque volumes of non-calcified plaques and overestimated the plaque volumes of calcified plaques (Schepis et al. 2010). Furthermore, interobserver variability ranged from 11% to 37%, casting doubt on the reproducibility of plaque quantification by MDCT (Leber et al. 2006; Schepis et al. 2010). Our interobserver variability for MDCT was 3.6%, perhaps a reflection of the relative immobility of SVGs and their larger size.
In contrast, there are no studies of SVG plaque quantification by MDCT or MRI. There is a recent study comparing dual-source CT and IVUS in assessing luminal area of SVG for stent sizing pre-intervention (Pregowski et al. 2011). They found good correlation ($r = 0.7$) but the lumen area were smaller on MDCT. Our data showed excellent correlation in assessing lumen area between CT and IVUS ($r=0.87$) and similarly found the lumen area by MDCT to be slightly smaller than IVUS (-0.6mm$^2$). However, the overestimation of vessel wall area resulted in a significantly larger overestimation (mean 66%) of plaque volume by MDCT while maintaining very good correlations with IVUS (Figures 4.3 & 4.4). This is most likely due to partial volume effects and presence of calcification in these old vein grafts.

The performance of MRI in plaque quantification was worse than MDCT. Although the correlation was excellent ($r=0.91$), there was gross overestimation (mean 255%) of the plaque volume compared to IVUS. The interobserver variability was also poorer compared with MDCT (9.9% v 3.6%).

4.3.1 Limitations

This is a small study of SVGs which were rather old and heavily diseased. In the acquisition of MRI images, we could only obtain orthogonal views for approximately 70% of the total lesion length due to vessel tortuosity, motion and limitations of breath hold. In contrast, MDCT was able to obtain a rapid volumetric data set, covering the entire lesion. Modern CT scanners with finer spatial resolution may result in better plaque quantification as partial volume effects are reduced. We used a 40Mhz IVUS catheter which was the predominant catheter in current use and offered good near field images. However, the use
of a 20Mhz catheter may have enabled better visualization in far field of some larger vein grafts.

4.4 CONCLUSION

Both MDCT and MRI have very good correlations with IVUS in SVG plaque quantification. However, there is significant overestimation of absolute plaque volume by MDCT and is much worse by MRI. Furthermore, MRI has poorer reproducibility and lesion coverage. Currently, non-invasive plaque quantification remains challenging and further research is warranted as technology continues to improve.
Chapter 5

Saphenous Vein Graft Plaque Characterization by Multi-Detector Computed Tomography with Histopathological Correlation of Embolic Debris During Intervention
The 10-year patency rate for saphenous vein grafts (SVG) has been reported to be as low as 50% (Cameron et al. 1995). Therefore, it is inevitable that a significant number of patients with SVGs will require some form of revascularization years after their original CABG. Repeat CABG is associated with an increased morbidity and mortality; hence the ability to revascularize percutaneously is an attractive option (Foster 1985). However, saphenous vein graft intervention remains challenging as there is a 10-30% risk of AMI by the ‘slow or no-reflow’ phenomenon despite a good procedural result at the site of intervention (de Feyter et al. 1993; Holmes et al. 1995; Hong, Mehran, et al. 1999). This phenomenon appears to be primarily mediated by the embolization of atheromatous material during stenting and has been reduced, but not eliminated, by distal protection devices (Baim et al. 2002; Stone et al. 2003). Therefore, predictors of atheromatous embolization pre-procedure could potentially stratify risk and assist in directing appropriate management strategies. There have been studies using x-ray angiographic findings to predict outcomes after saphenous vein graft intervention (Giugliano et al. 2005; van Gaal et al. 2007), but these are imperfect and require invasive angiography.

Multi-detector CT (MDCT) is a non-invasive technique that has been shown to accurately assess the degree of stenosis in saphenous vein grafts (Lau et al. 2006; Ropers et al. 2006) and correlates with intravascular ultrasound assessment of plaque burden in native coronary arteries (Achenbach et al. 2004; Leber et al. 2005; Moselewski et al. 2004). Thus, the potential exists for non-invasive assessment of saphenous vein grafts prior to intervention in symptomatic patients. We sought to investigate the relationship between saphenous vein graft plaque volume and density on CT, and the amount of atheroembolic material produced during vein graft intervention including histopathological features.
5.1 METHODS

This study was a prospective observational study involving consecutive patients referred for saphenous vein graft intervention at a single centre. The study protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and all subjects gave written informed consent. The study included 27 consecutive subjects with anginal symptoms in whom conventional coronary angiography revealed a significant lesion in a saphenous vein bypass graft suitable for PCI. All subjects underwent computed tomography angiography by a 16-detector row MDCT before SVG PCI. At the time of SVG PCI, a distal protection device (FilterWire EZ, Boston Scientific USA) was deployed and bare metal stents were used on all lesions. Unfractionated heparin was given as a bolus intravenously on table prior to intervention at a dose of 100 IU/kg. All patients were on aspirin and clopidogrel. This study excluded subjects presenting with an acute STEMI, severe left ventricular impairment (EF < 25%), lesions within 2.5 cm of distal anastamosis, lesions < 50% stenosis on angiography and inter-current illness or infection which may elicit a systemic inflammatory response.

5.1.1 Multi-detector computed tomography

All patients underwent CT angiography prior to SVG PCI utilising a 16-detector row CT (Sensation 16, Siemens Medical Solutions, Germany). Patients did not receive additional beta-blockade prior to scan. Image acquisition and scanner parameters were as described in Chapter 2.
Cross sections of the lesion perpendicular to the lumen were obtained at 1 mm intervals automatically utilizing Syngo Circulation software (Siemens Medical Solutions, Germany). The display setting used for lumen and plaque quantification followed previously published protocols. (Leber et al. 2005) After application of the appropriate window settings as described in Chapter 2, the lumen and vessel boundaries were manually traced (Figure 5.1) utilizing the Image Pro Plus software. The plaque volume was the result of the sum of these cross sectional areas.

Identification of coronary artery plaque and its calcific or non-calcific nature have been made utilizing existing published definitions (Achenbach et al. 2004; Leber et al. 2005). On every cross-sectional CT image, a region of interest (ROI) was placed over the atheroma. The ROI was the largest circle that could fit into the atheromatous area on the image without involving the lumen or outer vessel wall. The size of the ROI area (mm$^2$) and mean HU value was recorded from each ROI. The entire lesion HU was the mean of all the HU from the cross sectional images. A plaque with HU less than 60 was defined as lipidic, between 60 and 130 HU as fibrotic and above 130 to be calcific. These empiric definitions were based upon published data using the identical MDCT imaging system (Leber et al. 2004).
Figure 5.1 Correlation between invasive angiogram and MDCT. (A) Invasive angiogram of diseased SVG. (B) MDCT multi-planar reconstruction of same SVG with yellow line indicating plane of cross section. (C) MDCT showing SVG lesion in cross section. (D) Enlargement of image showing lumen (red) and vessel wall area (green).

5.1.2 Distal Protection Device.

Patients had the FilterWire EZ (Boston Scientific, USA) deployed during SVG PCI. In order to obtain the net embolic material weight, each FilterWire was processed by the same
senior laboratory technician who was blinded to all the data in this study. Specimens were
received in individual containers filled with formalin. All fluid was drained and specimen
left to stand for the same defined period of time before the FilterWire containing embolic
material was weighed intact on a calibrated scale (August Sauter GmbH D-7470, Albstadt
1-Ebingen, Germany). The FilterWire was dissected and all embolic material was
removed with a probe and flushed clean. The empty FilterWire was then blotted dry to
remove any remaining fluid before re-weighed on the same scale.

5.1.3 Histopathology

Once the material had been removed from the FilterWires, it was spun down using a
centrifuge (Heraeus Digifuge GL, Hanau, Germany). In order to fix the lipid component,
osmium tetroxide was applied and subsequently irrigated to remove excess osmium. The
material was then prepared in 15% bovine serum albumin and allowed to set before
embedding into paraffin blocks. These blocks were bisected vertically and stained for light
microscopy with haematoxylin & eosin, Picro Mallory (fibrin), Verhoeff-van Gieson
(elastin & collagen), and oil red ‘o’ (lipid). High resolution 12 megapixel TIFF images of
slides were obtained using a microscope and dedicated camera (Olympus BX41 and DP70,
Olympus Optical Co, Japan). These images were then analysed by an expert pathologist
and cardiologist utilizing semi-automated imaging software (Image-Pro Plus v5.1, Media
Cybernetics, USA) to identify plaque components: thrombus, fibrous, cholesterol clefts,
lipid and red blood cells (Figure 5.2). The absolute and percentage area of each component
were obtained.
Fig. 5.2 (A) Post Osmium fixed FilterWire embolic material stained with Oil-Red-O. (B): Semi-automated image analysis; green – plaque thrombus; yellow – lipid component; pink – red blood cells
5.1.4 **Statistical evaluation.**

All analyses were performed with SPSS 19.0 for Windows (IBM SPSS Statistics, USA). Data is represented as mean ± standard deviation or median and interquartile range as appropriate. The primary endpoint was embolic material weight from the FilterWires. Spearman’s rank correlation and Mann-Whitney tests were used for non-parametric data and unpaired t-tests used where appropriate. All p values are two-tailed with p<0.05 indicating statistical significance. The inter-observer variability of MDCT measurements was determined by coefficient of variance and Bland-Altman plots.

5.2 **RESULTS**

A total of 27 consecutive patients were enrolled when they presented with symptoms prior to SVG intervention. All underwent MDCT successfully and none were excluded due to image quality issues of MDCT. The mean heart rate of our patients was 63 (±11) bpm with 60% of them on β-blockers routinely. Two patients were excluded due to technical difficulties of the FilterWire device. Therefore, complete data was obtained in 25 patients with 27 lesions.

Table 5.1 summarizes the baseline patient characteristics. Angiographic and CT characteristics are summarized in Table 5.2.
### Table 5.1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.8 (±8.4)</td>
</tr>
<tr>
<td>Male</td>
<td>23 (92)</td>
</tr>
<tr>
<td>Age of SVG (years)</td>
<td>14.4 (±4.4)</td>
</tr>
<tr>
<td>ACS presentation</td>
<td>12 (48)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>13 (44)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>3 (12)</td>
</tr>
<tr>
<td>β-Blocker usage</td>
<td>15 (60)</td>
</tr>
<tr>
<td>HMG-CoA reductase inhibitor use (40-80 mg)</td>
<td>24 (96)</td>
</tr>
<tr>
<td>Other cholesterol lowering agents use</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Baseline Blood Results</th>
<th>Mean (Std Dev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>3.64 (±0.98)</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.94 (±0.24)</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.01 (±0.86)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.51 (±0.74)</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>77.6 (±27.0)</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>5.14 (5.51)</td>
</tr>
</tbody>
</table>

ACS indicates acute coronary syndrome; HMG-CoA, 3-hydroxy-3-methyl-glutaryl coenzyme A; hs-CRP, high sensitivity C-reactive protein; SVG, saphenous vein graft.
Table 5.2. Baseline Angiographic and CT Characteristics

<table>
<thead>
<tr>
<th>Angiographic Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion length (mm)</td>
<td>18.1</td>
</tr>
<tr>
<td>(IQR 12.1-26.5)</td>
<td></td>
</tr>
<tr>
<td>Total stent length</td>
<td>23.0</td>
</tr>
<tr>
<td>(IQR 18.0-28.0)</td>
<td></td>
</tr>
<tr>
<td>Reference vessel diameter (mm)</td>
<td>4.38</td>
</tr>
<tr>
<td>(±1.21)</td>
<td></td>
</tr>
<tr>
<td>Thrombus present</td>
<td>7.4%</td>
</tr>
<tr>
<td>Eccentricity</td>
<td>51.9%</td>
</tr>
<tr>
<td>Ulceration</td>
<td>29.6%</td>
</tr>
<tr>
<td>Calcification</td>
<td>14.8%</td>
</tr>
<tr>
<td>Angulation (&gt;45°)</td>
<td>11.1%</td>
</tr>
<tr>
<td>Target Vessel</td>
<td></td>
</tr>
<tr>
<td>SVG to LAD</td>
<td>22.2%</td>
</tr>
<tr>
<td>SVG to LCX</td>
<td>29.6%</td>
</tr>
<tr>
<td>SVG to RCA</td>
<td>48.2%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CT characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion length (mm)</td>
<td>18.0</td>
</tr>
<tr>
<td>(IQR 12.0-27.0)</td>
<td></td>
</tr>
<tr>
<td>Reference luminal diameter (mm)</td>
<td>4.46</td>
</tr>
<tr>
<td>(±1.05)</td>
<td></td>
</tr>
<tr>
<td>Plaque Volume (mm³)</td>
<td>358.78</td>
</tr>
<tr>
<td>(IQR 228.8-563.3)</td>
<td></td>
</tr>
<tr>
<td>Plaque HU density</td>
<td>72.0</td>
</tr>
<tr>
<td>(IQR 48.9-95.8)</td>
<td></td>
</tr>
<tr>
<td>Percentage atheroma volume</td>
<td>69.2%</td>
</tr>
<tr>
<td>(±9.6)</td>
<td></td>
</tr>
</tbody>
</table>

HU indicates Hounsfield unit; IQR, interquartile range; LAD, left anterior descending; LCX, left circumflex; RCA, right coronary artery; SVG, saphenous vein graft.
The median embolic material weight obtained from the FilterWire devices was 8 mg (IQR 4-13). Every FilterWire produced a yield of embolic material (range 1 – 33 mg). Table 5.3 summarizes the relationship between lesion characteristics, CT parameters, inflammatory markers, lipid profile and our primary outcome of embolic material weights.

Table 5.3. Relationship between various factors and embolic material weight

<table>
<thead>
<tr>
<th>Categorical Variables†</th>
<th>Embolic Material Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS presentation</td>
<td>5.4  8.0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8.0  8.0</td>
</tr>
<tr>
<td>Current smoker</td>
<td>6.0  8.0</td>
</tr>
</tbody>
</table>

ACS indicates acute coronary syndrome; * Continuous variables with Spearman’s correlations. † Categorical variables assessed by Mann-Whitney U test with median embolic material weight shown when the factor is present or absent.

We found that the baseline characteristics of age, sex, SVG age, smoking status, diabetic status or mode of presentation did not correlate with our primary endpoint.

The inter-observer variability of CT plaque volume as expressed by the coefficient of variance was 3.6%, with a Bland-Altman analysis showing a small difference of means of 33.8±57.7 mm³. Embolic material weight correlated with plaque volume by MDCT
The average Hounsfield Unit density of atheromatous plaque had a negative correlation with embolic material weight \((r = -0.439; p=0.025)\), with lower density plaques producing more debris (Figure 5.3B). Figure 5.4 demonstrates the tendency of lipidic plaques (HU<60) yielding more embolic material than fibrotic ones (HU 60-130) (mean 14.2 mg vs. 5.0 mg; \(p=0.0023\)). Only three patients had predominantly calcific plaques. Neither inflammatory markers nor lipid profiles correlated with the primary endpoint.

**Figure 5.3.** Correlation between embolic material weight and \((A)\) CT plaque volume \((B)\) CT plaque density.
Figure 5.4. Lipidic plaque (HU < 60) is represented by box-plot on the left yielding greater embolic material weight compared with fibrotic plaque (HU 60-130). Mann-Whitney U test; boxes represent interquartile range.

Table 5.4 display the findings of the histological analysis of embolic material and Table 5.5 summarizes the relationship of CT characteristics with histological plaque components. There were good correlations between plaque volume and the total area, lipid area and thrombus area. In addition, there was an inverse relationship between plaque density on CT and the amount of lipid found on histology ($r=-0.539; p=0.026$)
Table 5.4. Histopathological composition of embolic material captured

<table>
<thead>
<tr>
<th>Components</th>
<th>Present in % of samples</th>
<th>Mean area occupied (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>100%</td>
<td>0.6181</td>
</tr>
<tr>
<td>Lipid</td>
<td>69%</td>
<td>0.0552</td>
</tr>
<tr>
<td>Thrombus</td>
<td>65%</td>
<td>0.4088</td>
</tr>
<tr>
<td>Fibrous</td>
<td>50%</td>
<td>0.3041</td>
</tr>
<tr>
<td>Cholesterol clefts</td>
<td>23%</td>
<td>0.0204</td>
</tr>
</tbody>
</table>

Table 5.5. Correlation between CT plaque volume with histology components

<table>
<thead>
<tr>
<th>Histopathology components</th>
<th>r value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total area</td>
<td>0.514</td>
<td>0.007</td>
</tr>
<tr>
<td>Lipid area</td>
<td>0.613</td>
<td>0.009</td>
</tr>
<tr>
<td>Thrombus area</td>
<td>0.740</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol area</td>
<td>0.771</td>
<td>0.07</td>
</tr>
<tr>
<td>Red blood cells area</td>
<td>-0.07</td>
<td>0.72</td>
</tr>
<tr>
<td>Fibrous area</td>
<td>0.51</td>
<td>0.07</td>
</tr>
</tbody>
</table>
We observed one cardiovascular death and two peri-procedural AMI in our cohort. The death was from an AMI five days after SVG intervention. The two patients with peri-procedural AMI were the result of ‘slow flow’ during SVG intervention. Table 5.6 displays the predictors of the combined outcome of AMI and death, as analysed by Mann-Whitney U and unpaired t-tests. The group with complications had higher plaque volumes and greater percentage atheroma volume as assessed by CT.

Table 5.6. Comparison between patients with and without complications

<table>
<thead>
<tr>
<th></th>
<th>AMI/Death</th>
<th>Well</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDCT plaque volume (mm$^3$) *</td>
<td>1480.0</td>
<td>309.2</td>
<td>0.017</td>
</tr>
<tr>
<td>Plaque HU density *</td>
<td>37.0</td>
<td>78.6</td>
<td>0.123</td>
</tr>
<tr>
<td>Age (years) †</td>
<td>67.8</td>
<td>67.9</td>
<td>0.985</td>
</tr>
<tr>
<td>SVG age (years) †</td>
<td>12.5</td>
<td>14.6</td>
<td>0.428</td>
</tr>
<tr>
<td>CT Percentage Atheroma Volume †</td>
<td>83.23%</td>
<td>67.42%</td>
<td>0.005</td>
</tr>
</tbody>
</table>

AMI indicate acute myocardial infarction; HU indicates Hounsfield unit; SVG, saphenous vein graft.

* Assessed by Mann-Whitney U test. † Assessed by unpaired t-test.
5.3 Discussion

Interventions in saphenous vein grafts compared to native vessels are associated with an increase in major adverse cardiac event rates (MACE). These adverse events are associated with degree of graft degeneration, vessel size and lesion length however adverse events can still occur in the absence of these standard angiographic markers (Stone 2005; Stone et al. 2003). Our study demonstrates that not only is MDCT able to non-invasively quantify and characterize atheroma in vein grafts, but it provides information regarding the risk of atheroma embolization prior to any invasive procedure. Given that plaque embolization may still occur despite the use of distal protection devices, additional information may be useful when evaluating treatment options.

The ability of MDCT to reliably measure plaque area, degree of stenosis and remodelling index has been studied previously in native coronary arteries (Hoffmann, U et al. 2006; Moselewski et al. 2004), including the characterisation of the lipidic or calcific nature of plaques when compared to intravascular ultrasound (Achenbach et al. 2004; Leber et al. 2004). To date our study is the first to evaluate plaque morphology utilizing MDCT in relation to SVGs and associated high risk PCI. Although there have been studies in SVGs comparing accuracy of 4, 16 and 64 detector CT to coronary angiography in relation to degree of stenosis, to date, our study is the only one to explore plaque volume of entire lesions with relation to embolic material weight.

A recent study using FilterWire in SVG PCI, did not find any correlation between the uniplanar area of the FilterWire which was covered with embolic material, and a multitude
of angiographic, procedural and clinical variables (van Gaal et al. 2007). In contrast, by using embolic material weight as the endpoint, we were able to better define the degree of plaque embolization. A larger study of 194 patients undergoing a mixture of coronary and vein graft PCI utilizing FilterWire devices, concluded that longer stent lengths and final TIMI flow < 3 were predictors of ‘visually significant’ debris greater than 1 mm (El-Jack et al. 2007). A recent large study of nearly 4000 patients derived from pooled data from five randomized trials and one registry found two angiographic markers to predict 30-day MACE (Coolong et al. 2008). These two novel markers measured by quantitative coronary angiography were estimated plaque volume (derived from a mathematical formula) and SVG degeneration score. The correlation between distal embolization and plaque volume by CT in our study lends further credit to the concept of a diseased vein graft with greater plaque burden being of higher risk.

Unlike native coronaries, plaques in SVG tend to be cholesterol rich with relatively low calcium content (Gorog et al. 2005), yielding debris largely consisting of necrotic tissue and fibrin (Grube et al. 2002). Therefore, one might hypothesize that cholesterol rich, soft lipidic plaque of vein grafts would be more likely to embolize than harder fibrotic plaque during intervention. Our data showed a statistically significant negative correlation between HU density and embolic material weight, suggesting denser plaques will yield less embolic material. It is difficult for current CT technology to reliably distinguish lipidic from fibrotic plaque. However, our data demonstrate a tendency for lipidic plaques to produce more embolic material compared to fibrotic ones (Figure 5.4), albeit with some overlap between the two groups. Histopathological analysis of the embolic material revealed that larger plaques on CT produced debris containing more lipid and thrombus. A lower plaque density on CT also resulted in debris containing more lipid content. This
lends further credit to the idea that larger and lower density plaques which are likely to be lipid rich have a greater tendency to embolize during SVG intervention.

Although our study cohort is a small one, we observed a cardiovascular death and two peri-procedural AMI. This translates to a MACE rate of approximately 12%, which is comparable to large trials of 9-21% (Baim et al. 2002; Stone et al. 2003; Stone et al. 2002). No firm conclusions can be made from such a small sample, however we found a statistical difference with larger plaque volumes and greater CT percentage atheroma volume in the group that experienced the complications.

5.3.1 Limitations of Study.

This is a small study, and as such we do not have the ability to assess the independence of the factors associated with embolic material weight with multivariate logistic regression. However, the potential to provide predictive information about outcomes in SVG intervention non-invasively with MDCT would be an exciting advance. Further work to assess the potential additive risk information provided by plaque characterisation with MDCT is needed, but our preliminary data supports its potential.

To date there are no validation studies of plaque volume in SVG using MDCT. Plaque volume quantification has been investigated in the native coronary arteries but not saphenous vein grafts. These studies in native coronary arteries utilizing 16 detector CT have shown good correlations between MDCT and IVUS for the estimation of plaque volume (Achenbach et al. 2004).
5.4 Conclusion

Plaque volume by MDCT and average HU density of plaque were significant correlates of embolic material weight captured by the FilterWire device during saphenous vein graft intervention. Histopathology analysis of the debris revealed greater lipid and thrombus components from plaques that were larger in CT volume. Furthermore, lower density plaques on CT produced debris with more lipid component. We have shown for the first time that MDCT as a non-invasive tool can provide information regarding atheroembolic risk prior to intervention. Further study is warranted to investigate the role of MDCT in future risk assessment of saphenous vein graft intervention.
Chapter 6

MRI PLAQUE ASSESSMENT OF CAROTID ARTERY STENOSIS

PRIOR TO INTERVENTION WITH HISTOPATHOLOGICAL CORRELATION
The treatment of severe carotid artery stenosis has traditionally been carotid endarterectomy (CEA). Over the last two decades carotid artery stenting (CAS) has showed great promise as a less invasive alternative but has provided equivocal results at best. Whilst there are many advantages to CAS with regards to smaller wound, use of local anaesthetic, avoidance of injury to cranial nerves and other neck structures, randomized trials have shown a slightly worse short-term outcome, largely driven by a higher incidence of non-disabling stroke (Meier et al. 2010). The main cause of these strokes is probably distal embolization of atheromatous material during CAS. The use of distal protection devices (DPD) have reduced this risk but does not eliminate it completely (Kastrup et al. 2006). Furthermore, significant reduction in antegrade flow or ‘slow flow phenomenon’ during CAS can result in significantly worse outcome of stroke and death at 30 days (Casserly et al. 2005).

The ability to identify patients who are at increased risk of embolic complications prior to CAS would be helpful to guide therapeutic decisions. It is uncertain if the carotid plaque volume and morphology has any impact on the amount of embolic debris generated during CAS. A pre-procedural MRI scan of the carotid lesion may provide useful information on the atherosclerotic plaque volume and characteristics. MRI has been shown to be accurate and reproducible method of plaque quantification (Touze et al. 2007). Its ability to characterize different plaque components eg. lipid-rich, fibrous, calcium and intraplaque haemorrhage have also been well documented (Cai, JM et al. 2002; Kampschulte et al. 2004; Yuan et al. 2001). Therefore, we sought to conduct a pilot study to determine if plaque volume and morphology on a pre-procedural MRI scan of carotid lesions may provide insights on the amount of embolic debris generated and the subsequent histological analysis of such debris.
6.1 METHODS

Consecutive patients (n=10) with symptomatic severe carotid stenosis (>70% on previous ultrasound or CT) were enrolled in this study. Patients were excluded if they could not give informed consent, deemed unsuitable for CAS, complete occlusion, unable to tolerate anti-platelets (aspirin, clopidogrel) or had contra-indications for MRI scanning.

All underwent MRI scanning prior to CAS using a 1.5 Tesla clinical MRI system (Siemens Sonata, Germany) and dedicated carotid coil. Multi-contrast images were obtained (TOF, T1W, PDW and T2W) at the common carotid just below the bifurcation and 6 consecutive images of 3mm slice thickness (no gaps) starting at the bifurcation, covering the internal carotid artery. Parameters for the sequences were (i) 2D-TOF (TR/TE 28/6.5 ms, flip angle 50) (ii) T1W black blood turbo spin echo (TR/TE 900/7.7 ms) (iii) PDW turbo spin echo (TR 3RR, TE 7.7 ms) (iv) T2W turbo spin echo (TR 3RR, TE 69 ms). All images were cardiac gated, field of view [FOV] 12 cm, thickness 3mm, 256 x 256 matrix, number of excitations 2. Voxel size was 0.46 x 0.46 x 3.0 mm.

Images were converted from DICOM to TIFF format and analysed offline using dedicated imaging software (Image-Pro Plus v5.1, Media Cybernetics, USA). The lumen and outer vessel borders were traced manually at each level. The plaque area was the vessel wall area (VWA) minus the luminal area. The plaque volume was the summation of the plaque areas along the lesion. Plaque characteristics were determine by comparing the relative intensity of the signal to the adjacent sternocleidomastoid muscle on different weighted contrast
images as described in Chapter 2. They were categorised into (i) calcific (ii) fibrotic (iii) lipid rich – necrotic core or (iv) intra-plaque haemorrhage.

Carotid artery stenting was carried out by interventional radiologist or vascular surgeon expert in the procedure. Intravenous heparin (100 IU/kg) was given prior to the procedure. A guiding catheter was placed in the common carotid artery and angiograms were obtained. The distal protection device, Angioguard (Cordis, USA) was used in all patients prior to stenting using stents of their choice. Post dilatation was carried out in all patients before removal of the Angioguard device. Final angiograms were obtained at the conclusion of the procedure.

The Angioguard devices were immediately retrieved and kept in formalin for transport to pathology for preparation and analysis. The embolic material was weighed and prepared as stated in Chapter 2. Multiple stains were performed to demonstrate different plaque components and high resolution photomicrographs were obtained for image analysis using Image-Pro Plus v5.1 (Media Cybernetics, USA) as outlined in Chapter 2 (Figure 6.1).

Statistical analysis was conducted with SPSS 19.0 for Windows (IBM SPSS Statistics, USA). Simple linear regression was performed to analyse the relationship between embolic material weight and MRI plaque volume. The amount of embolic material produced by different plaque types as characterized by MRI was compared using Kruskal-Wallis test. A p-value of less than 0.05 was deemed significant.
Figure 6.1. High resolution image of one specimen (top panel) and overlayed analysis (bottom panel).

Thrombus is showed in green; lipid in yellow and red blood cells in pink.
6.2 Results

All patients (age 72±7.7 years, male 64%) underwent MRI and CAS successfully. There were no complications during CAS. Every Angioguard device managed to capture some debris (4.4 – 20.2 mg; mean 13.8 ± 4.8 mg). The MRI plaque volume ranged from 291mm$^3$ to 1485mm$^3$ (mean 609 ± 343 mm$^3$). There was no relationship between the plaque volume on MRI and subsequent amount of embolic material captured (Figure 6.2). There were also no relationships between age, sex, serum cholesterol level, smoking status and diabetes on the amount of embolic material captured.

![Correlation of MRI plaque volume & embolic material captured](image)

Figure 6.2. Correlation of MRI plaque volume and embolic material weight.

Plaque characteristics determined by MRI revealed the plaques were calcific (n=3), fibrous (n=6) and lipid rich/necrotic core (n=1) (Figure 6.3). There were no lesions with intra-plaque haemorrhage in our cohort. There was no relationship between the plaque
characteristics on MRI and the amount of embolic material collected (Kruskal-Wallis test; \( p = 0.29 \)) during CAS (Figure 6.4). Furthermore, there were no significant differences in quantity of individual plaque components found on histopathology between the three MRI plaque types.

**Figure 6.3.** Multi-weighted MRI images of left carotid artery; **SCM** denotes sternocleidomastoid muscle; \# denotes external carotid artery, * denotes internal carotid artery, arrows show the lipid rich plaque of ICA. In comparison to SCM: A) TOF – isointense; B) T1W – isointense; C) PDW – isointense to hypointense; D) T2W – hypointense.
Histopathology analysis revealed every specimen contained some free red blood cells and 67% contained organized thrombus. Only one specimen showed fibrous tissue and another showed cholesterol clefts. About one fifth of specimens contained lipid components (Table 6.1). There was no relationship between plaque characteristics (calcific, fibrotic, lipidic) on MRI and the subsequent histopathology make-up of the embolic material captured during CAS.
Table 6.1. Histopathology analysis of embolic material captured.

<table>
<thead>
<tr>
<th>Plaque components</th>
<th>Presence in specimens (%)</th>
<th>Mean area occupied (mm²)</th>
<th>Percentage of plaque area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrous</td>
<td>11.1</td>
<td>0.0082</td>
<td>0.66</td>
</tr>
<tr>
<td>Thrombus</td>
<td>66.7</td>
<td>0.1212</td>
<td>23.81</td>
</tr>
<tr>
<td>Lipid</td>
<td>22.2</td>
<td>0.0002</td>
<td>0.03</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>100</td>
<td>0.4562</td>
<td>75.46</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>11.1</td>
<td>0.0002</td>
<td>0.04</td>
</tr>
<tr>
<td>Total components</td>
<td>-</td>
<td>0.59</td>
<td>-</td>
</tr>
</tbody>
</table>

6.3 Discussion

In this pilot study, we investigated if any relationship exists between plaque volume and characteristics on pre-procedural MRI and subsequent amount of embolic material captured during CAS. We also conducted histopathological analysis of such debris to determine any correlations between imaging parameters and histopathology components. To our knowledge there have been no studies using carotid MRI to predict subsequent embolic events or amount of embolic material debris captured.

We were not able to find any correlations between plaque volume on MRI and subsequent amount of embolic material captured by the Angioguard device. Similarly plaque characteristics on MRI did not predict the amount or histopathological nature of the embolic material captured. The underlying reason is likely to be multifactorial but a significant limitation of this study is the small sample size. There was only one patient with
features of lipid-rich / necrotic core on MRI and none with intraplaque haemorrhage. This may reflect the relatively ‘stable’ plaques of carotid arteries in general or elective nature of our cohort. Other reasons for our negative findings may include limitations in image analysis, procedural factors and deficiencies in distal protection devices.

The plaque quantification techniques which we employed have been validated and published previously (Helft et al. 2002; Helft et al. 2001; Saam et al. 2005). There is an inherent limitation to accurate volume measurements due to the MRI slice thickness of 3mm employed. A much thinner slice eg. 1mm would theoretically yield a more accurate plaque volume however there are technical and practical limitations as it would render the entire study prohibitively long to complete. In any case, the best achievable slice thickness for in-vivo carotid arteries using 1.5T systems is 2mm and only a few images were obtained spaced widely apart for plaque characterization and not continuous volume measurements when studied previously (Yuan et al. 2001).

Evidence of embolism during carotid intervention has been demonstrated by transcranial Doppler monitoring and post-procedural brain MRI (Markus 2000; Schluter et al. 2003). Ischaemic lesions in the brain can be detected on standard MRI study in about 11% of patients post CAS in one study (van Heesewijk et al. 2002). The positive predictive value for lesions detected by MRI which is clinically significant is 45% (Hellings et al. 2006). However, using diffusion weighted imaging (DWI) which is a very sensitive MRI enhancement technique that detects acute ischaemia, new lesions are found in up to 23-57% of patients (Hellings et al. 2006). These lesions were small and usually asymptomatic. This was supported by another study which performed follow up MRI and DWI scans at 6
months and found most DWI lesions have disappeared, suggesting this type of acute ischaemia may be reversible (Hauth et al. 2005).

The impact of embolism has been reduced but not eliminated by the use of distal protection devices. In a recent meta-analysis, the use of distal protection devices reduced the incidence of DWI lesions on MRI from 45% to 33% (Schnaudigel et al. 2008). Clearly these devices are imperfect – they do not capture all of the embolic debris which may result from the intervention. Debris particles smaller than pore size of the device, incomplete apposition of the device against vessel wall, large amounts of debris overwhelming the device and inadvertent dislodgement of debris prior to device deployment are some of the possible reasons (Casserly et al. 2005; Hellings et al. 2006). Any of those factors may have influenced the amount of embolic debris captured by the Angioguard device in our study. However, in a large study of 550 patients, it was found that the majority of embolism occurred after the distal protection device has been deployed, during stent deployment and post-dilatation (Ackerstaff et al. 2005). We also did not perform MRI brain scans to document and perhaps investigate if there is an inverse relationship between the number of ischaemic lesions in the brain and the amount of the embolic debris captured.

In this study, we used the Angioguard device as the operators were most familiar with its use and to ensure uniformity. A recent study found there was a significant difference in the efficacy of various embolic protection devices in trapping the embolic debris (DeRubertis et al. 2007). Of the four devices studied, the Angioguard device was second best, trapping almost half the mean number of particles compared to top performer, EPI FilterWire
(Boston Scientific, Natick, MA, USA). This may be a factor in the poor correlation we found between plaque volume on MRI and embolic material weight in our study.

While there have been studies analysing the embolic debris captured, almost all have commented on sizing of particles and only brief description of its composition (Angelini et al. 2002; DeRubertis et al. 2007; Whitlow et al. 2002). None have measured the quantity of each component in the embolic material. In one study on Angioguard devices, the particles trapped were commonly greater than 300 μm, but particles smaller than the size of the filter pore (100 μm) have been found occasionally (Angelini et al. 2002). They described finding soft acellular and amorphous material with lipid rich macrophages and cholesterol clefts on light microscopy. We are unique in documenting how often each component (red blood cells, thrombus, lipid, fibrous tissue, cholesterol clefts) is found for any given device, the percentage and absolute area which it occupies of that device.

6.4 Conclusion

In this pilot study we were unable demonstrate any correlations between plaque volume and characteristics determined by MRI and embolic material weight captured by a distal protection device during carotid artery stenting. A major limitation is the small sample size and paucity of lipidic plaques. Using specialized imaging software and histopathology techniques, we were able to uniquely measure various components of plaque debris and their percentage contribution to the embolic material.
Chapter 7

DISCUSSION AND SUMMARY
This thesis investigates the utility of MDCT and MRI in plaque assessment prior to interventional procedures. The rapid development of MDCT in particular has led to increasing clinical use of MDCT around the world. Most initial studies are focused on validation of this new modality against the traditional gold standard of invasive angiography. We have taken this further in not only assessing the accuracy of MDCT and MRI but explored relationships between plaque characterization, embolization and histopathological findings.

We provided a broad review of the literature of atherosclerosis and discussed the concept of vulnerable plaque. The current invasive and non-invasive imaging modalities for atherosclerotic plaques were presented, with particular emphasis on MDCT and MRI. We also reviewed the areas of SVG disease and carotid artery disease along with issues specific to percutaneous interventional treatments. Chapter 2 outlines the methodologies employed in our studies and image analysis.

In Chapter 3, we first studied angiographic predictors for impaired flow during SVG intervention. We found significant correlations between the reference vessel diameter, number of stents used and estimated plaque volume, and the amount of embolic material captured by the distal protection device. We showed that the quantity of debris produced during SVG intervention has a bearing on flow as determined by TIMI frame count. The importance of using a distal protection device during SVG intervention is emphasized by our results as there was worsening of the TIMI frame count in larger plaques. This reinforces the knowledge that distal protection devices may not capture 100% of the debris and can reduce the embolic complications but cannot eliminate it. These findings strengthens the concept of ‘slow flow’ may be largely determined by plaque embolization.
During PCI. Larger plaque burdens subjected to greater mechanical manipulation by way of multiple stents will produce more embolic debris, which in turns impacts on flow within the vessel.

We then begin our investigation of non-invasive imaging by determining the accuracy and reproducibility of MDCT and MRI in plaque quantification against the gold standard of IVUS. While we found excellent correlations between the modalities and IVUS, the absolute volumes were overestimated by MDCT by 66% and more so by MRI by 255%. The agreement was excellent for luminal dimensions but poor for vessel wall area, hence impacting on the overall plaque volumes. Reproducibility was good for both modalities but much better for MDCT. MDCT was the only modality where entire lesions were adequately visualized as MRI image acquisition is difficult in tortuous vessels. Therefore, it seems both MDCT and MRI cannot replace IVUS in determining absolute plaque volumes but can reliably provide good correlations.

In Chapter 5, we used MDCT to assess plaques prior to SVG intervention. We found larger plaque volumes will result in larger amounts of embolic material captured by the distal protection device. We also found that lower plaque densities which may represent more lipidic or ‘soft’ plaques produced more embolization. This was confirmed when we conducted histopathological analysis of the embolic material and found that larger plaques and lower density plaques contain more lipid components. Although we must proceed with caution interpreting outcomes in small studies, the three patients who suffered adverse events had significantly greater plaque volume on MDCT compared with those who did not. This is the first demonstration that plaque composition along with plaque burden is associated with plaque embolization during SVG intervention.
Our final original chapter investigates the use of multi-weighted MRI imaging in characterizing carotid plaques prior to stenting. We attempted to explore if there is any associations between plaque type and volume with subsequent amount of embolic material captured by the distal protection device. Our results were disappointing but there were many factors which were different to our SVG study. Firstly, we were now dealing with arteries rather than friable saphenous veins, which may have different embolic tendencies. The use of a different type of distal protection device from that used in our SVG study means that the efficacy in capturing embolic debris will also be different. These factors compound the inherent limitation of our small numbers.

In this thesis we have conducted novel studies which contribute to our knowledge of non-invasive imaging and factors associated with plaque embolization during percutaneous intervention. The area of imaging is evolving rapidly with MDCT in particular making improvements in speed, coverage and resolution. Clearly further research is needed and in future non-invasive imaging may have a role in assessing not only stenosis severity but incremental risk prediction for adverse events.
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