THE CONTRIBUTION OF INFLAMMATORY MECHANISMS TO MICROVASCULAR INJURY IN CORONARY ANGIOPLASTY

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by

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Abstract
Coronary angioplasty is established as the most common form of revascularisation in IHD, yet despite major advances, failure of microvascular revascularisation is associated with poor prognosis. Inflammation is important in the natural progression of atherosclerosis from early stages through to progression to plaque rupture and acute myocardial infarction. In addition inflammatory mechanisms have a role in mediating adverse clinical outcomes during coronary reperfusion and revascularisation, although the underlying mechanisms remain unknown.

The objective of this research was to assess the microvascular response to percutaneous coronary intervention (PCI) in patients with stable angina, and this study aimed to assess the relationship between changes in microvascular function in response to PCI with markers of systemic inflammation and endothelial activation. Also we aimed to detect localised myocardial inflammation in response to PCI in those patients who have microvascular injury following PCI.

We measured fractional flow reserve (FFR), coronary flow reserve (CFR) and index of microcirculatory resistance (IMR) in both the vessel undergoing PCI and an adjacent vessel, before and after PCI. We assessed the inflammatory response to PCI and the relationship between markers of systemic inflammation and microvascular function. A subgroup of 9 patients underwent radiolabelling of leukocytes and myocardial SPECT scans before and after PCI to detect areas of localised inflammation. A total of 39 patients were included in the study.

Patients demonstrated a heterogenous response on IMR following PCI. There was an increase of microvascular resistance in the vessel undergoing PCI in a third of subjects. There was a reduction of IMR in the reference vessel in response to PCI in the target vessel (23.7[15.8-31.4] vs. 17.9[12.4-24.1] n=34 $p<0.01$) Basal inflammation was not predictive of microvascular dysfunction but the inflammatory response as measured by CRP and ET-1 was associated with microvascular dysfunction ($R=0.42$ $p<0.05$, $R=0.34$ $p<0.05$ n=35) respectively. A low pre procedural IMR was strongly predictive of an increase in microvascular resistance post PCI ($R=0.73$ $P<0.001$). Finally no areas of increased inflammation were detected on myocardial SPECT scanning. In summary it is the inflammatory response rather than basal levels of inflammation which seem to be

associated with microvascular dysfunction and we see a novel reduction in IMR in the reference vessel in response to PCI in the target vessel.

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B Patel, L.Smith, P.Browning, G. Hart, M.Fisher. PCI causes reduction in microvascular resistance in the adjacent vessel bed. *J. Am. Coll. Cardiol. 2009;53;A92 (2523-824)*

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Glossary

ACD	Acid citrate dextrose
ACH	Acetylcholine
ACS	Acute coronary syndrome
ACE	Angiotensin-II converting enzyme
AMI	Acute myocardial infarction
ARMYDA-CAM	Atorvastatin for reduction of myocardial damage during angioplasty-cell adhesion molecules
BMI	Body mass index
BMS	Bare metal stent
cAMP	Cyclic AMP
CABG	Coronary artery bypass grafting
CARE	Cholesterol and recurrent events
CAM	Cellular adhesion molecule
CCS	Canadian cardiovascular society
CFI	Collateral flow index
CFR	Coronary flow reserve
CHD	Coronary heart disease
CK	Creatine kinase
CKMB	Creatine kinase myocardial bound
COPD	Chronic obstructive pulmonary disease
CRF	Chronic renal failure
CRP	C- reactive protein
CS	Coronary sinus
CT	Computed tomography
CTO	Chronic total occlusion
CV	Co-efficient of variance
DE	Distal embolisation
DES	Drug eluting stent
DPD	Distal protection device
EDHF	Endothelial derived hyperpolarising factor
EDRF	Endothelial derived relaxing factor
e-NOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1

ETT	Exercise tolerance test
ELISA	Enzyme-linked immunosorbent assay
FMD	Flow mediated dilation
FFR	Fractional flow reserve
FDG	Fludeoxyglucose
FITC	Fluorescein isothyocyanate
Gd-DTPA	Gadolinium-diethylenetriamine pentaacetic acid
GTN	Glyceryl trinitrate
HDL	High density lipoprotein
HITS	High intensity signal?
HMPAO	Hexamethylpropyleneamine oxime
HsCRP	High sensitivity CRP
I-CAM	Intra cellular adhesion molecule
IHD	Ischaemic heart disease
IL-6	Interleukin-6
IMR	Index of microcirculatory resistance
ISAR	Innovative stratification of arrythmia risk
JUPITER	Justification for the use of statins in primary prevention:an intervention trial evaluating rosuvastatin
K^+_{ATP}	Potassium adenosine triphosphate
LAD	Left anterior descending
LDL	Low density lipoprotein
LMS	Left main stem
LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVEDP	Left ventricular end diastolic volume
MAC-1	Macrophage-1 antigen
MACE	Major adverse clinical events
MCP-1	Monocyte chemo attractant protein
M-CSF	Macrophage colony stimulating factor
MI	Myocardial infarction

MFI	Mean fluorescence intensity
MR_V	Myocardial microcirculatory resistance assessed with doppler
MPG	Myocardial perfusion grade
MR	Myocardial resistance
MRI	Magnetic resonance imaging
NO	Nitric oxide
NSTEMI	Non-ST elevation myocardial infarction
PCI	Percutaneous coronary intervention
P_d	Mean distal coronary pressure
P_a	Mean arterial pressure
P_w	Mean wedge pressure
P_v	Venous pressure
PE	Phycoerythrin
PET	Positron emission tomography
PIL	Patient information leaflet
PKC	Protein kinase C
PLC	Phophlipase C
PMI	Peri-procedural myocardial injury
PSGL-1	P-selectin glycoprotein ligand-1
QCA	Quantitative coronary angiography
ROI	Region of interest
SPECT	Single photon emission computed tomography
STEMI	ST elevation myocardial infarction
Q_{myo}	Myocardial flow
Q_{coll}	Collateral flow
Q_{cor}	Coronary flow
RVEDP	Right ventricular end diastolic pressure
SSC	Side scatter
TIMI	Thrombolysis in myocardial infarction
TFG	TIMI flow grade
TFC	TIMI frame count
TMR	Total myocardial resistance

T_{mn}	Mean transit time
$T_{mn(hyper)}$	Mean transit time at hyperaemia
$T_{mn(rest)}$	Mean transit time at rest
TnT	Troponin T
USPIO	Ultra small paramagnetic iron oxide
V	Volume
V-CAM	Vascular cellular adhesion molecule
VSMC	Vascular smooth muscle cell
WBC	White blood cell

Chapter 1 Introduction

1.1 Coronary artery disease

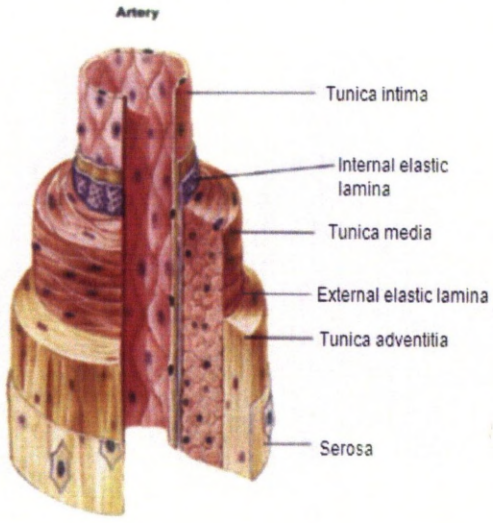
1.1.1 Significance of coronary artery disease

Cardiovascular disease causes more than 1 in 3 deaths in the UK accounting for almost 200,000 deaths each year (2008b). The main causes are coronary heart disease (CHD) and cerebrovascular disease. CHD causes around 94,000 deaths in the UK each year and is the most common cause of premature death. About one fifth of premature deaths in men and one in ten in women are caused by CHD (Allender , 2008). CHD most commonly presents as acute myocardial infarction (MI) or angina, approximately 146,000 MI occur per year in the UK (Volmink *et al.*, 1998) and nearly 2 million people in the UK either suffer from angina or have had angina at some stage in their lives. The prevalence of CHD increases with age, 1 in 3 men and 1 in 4 women over age of 75 are affected by CHD (2008a). The high prevalence of this disease results in a massive economic burden costing the UK economy a total of 9 billion pounds a year, of which 3.2 billion pounds in health care costs and the remainder in loss of productivity and informal care of people with CHD (Allender , 2008). Although there has been a significant reduction in mortality from CHD over the last decade due to better access to diagnosis and revascularisation services, the prevalence of CHD continues to rise due to factors such as the increase in the ageing population and the increase of type II diabetes and obesity (DH Coronary Heart Disease Policy Team, 2008).

1.1.2 Structure of the coronary artery

The normal coronary artery consists of three layers; the intima, media and adventitia (Figure 1.1). The outermost layer is known as the adventitia and consists, mainly of collagen fibrils, tiny blood vessels, vaso vasorum (which supply the wall of the artery), nerve endings, fibroblasts and mast cells. Within the adventia lies the media, which is separated from the adventitia by the external elastic lamina. The media provides the vessel with elastic properties and consists of concentric layers of smooth muscle cells with layers of extracellular matrix composed of compound high in elastin. The media is separated from the innermost layer known as the intima by the internal elastic lamina. The intima made up of a surface layer of endothelium over a basement membrane consisting of type four collagen, laminin, fibronectin and other extracellular matrix molecules (Wight, 1989). Deep to the basement membrane is the intimal layer, this consists of sub endothelial connective tissue and sparse smooth muscle cells.

Figure 1.1 Structure of the coronary artery.



Schematic representing the different layers of the coronary anatomy.

Adapted from Fox and Ira, Human Physiology Sherwood publishers 11th Edition.

1.1.3 The role of the endothelium

The endothelium is a single cell layer that lines the normal arterial tree which would cover approximately 1000m^2 if opened out into a single sheet (Augustin *et al.*, 1994). This layer of cells is capable of sensing and reacting to mechanical forces within the lumen such as shear stress and producing and releasing various vasoactive compounds to maintain the equilibrium of vascular tone, as well as having potent anticoagulant

properties. Clear evidence that the endothelium is a source of biologically active mediators was provided following the discovery of endothelium-derived-relaxing-factor (EDRF). The biological effects of EDRF were subsequently shown to be due to nitric oxide (NO) (Palmer *et al.*, 1987;Palmer *et al.*, 1988b), and it became clear that NO was crucially important in the maintenance and regulation of arterial tone (Furchgott and Vanhoutte, 1989). Since the ground breaking discovery of NO a whole range of vasoactive substances have been discovered as originating from the endothelium which serve to regulate vessel tone. These include substances which have vasoconstricting properties, such as endothelin-1(ET-1), angiotensin, thromboxane A2 and prostaglandin H2, as well as vasodilatory substances such as prostacyclin, bradykinin and endothelial derived hyperpolarising factor (Drexler and Hornig, 1999;Mombouli and Vanhoutte, 1999;Verma and Anderson, 2002). Since these mediators have opposing properties the net effect in a finely balanced and healthy endothelium is the maintenance of vascular relaxation. In addition to the regulation of tone, the endothelium performs a variety of other functions which maintain arterial homeostasis, this is due to the ability of the endothelium to produce a non adherent surface which resists the attachment of leukocytes and platelets by the balance of pro and anticoagulant factors (Edelberg *et al.*, 2001). Endothelial cells express a combination of pro-coagulant and anti coagulant properties. Pro-coagulant factors such as plasminogen activator tissue factor and Von Willebrand factor have to be in equilibrium with anticoagulant factors such as heparan sulphate, thrombomodulin, prostacyclin and tissue type plasminogen activator. It is this equilibrium between pro

and anticoagulant activity that allows maintenance of the flow of blood without formation of thrombus. However when the endothelium becomes damaged or dysfunctional then the balance between factors shifts in the favour of a state which promotes coagulation (Schafer, 1997).

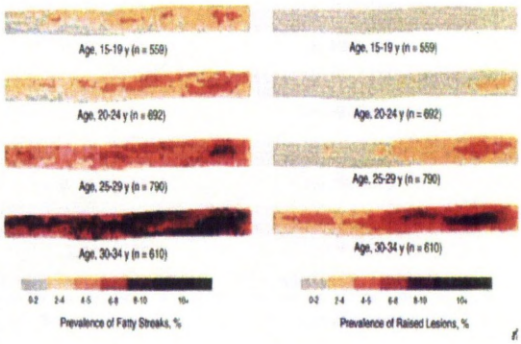
In addition to the vasoactive and anticoagulant function of the endothelium there is also a structural function whereby the endothelium is able to secrete its own basement membrane and other elements of the extra-cellular matrix, including proteoglycans and elastic fibres (Wight, 1995;Newby and Zaltsman, 1999). The endothelium also acts as a barrier between the circulating cells and the subendothelial space, however there is some degree of permeability such that certain cell types may migrate through tight junctions between endothelial cells known as desmosomes. The function of desmosomes is predominantly to prevent diffusion of macromolecules or the migration of cells through the endothelium (Rubin, 1992;Schneeberger and Lynch, 1992). Any insult or injury to the endothelium affects the level of permeability of the endothelium, which is thought to be one of the earliest change in the genesis of atherosclerosis (Ross, 1995)(Figure 1.3).

1.4 Pathophysiology of atherosclerosis

Atherosclerosis has only been named as a pathological entity within the last century although there is evidence of the disease process as far back as over three thousand years ago in the remains of Egyptian mummies (Shattock, 1909). Great advances have

been made in understanding the pathogenesis of atherosclerosis in the last 60 years. Post mortem studies of young soldiers killed in action in the Korean war described the presence of atherosclerosis in coronary arteries of previously healthy men (ENOS *et al.*, 1953;Enos *et al.*, 1986). The fatty streak is often seen in even younger subjects of children and infants (Schwartz *et al.*, 1967). There seems to be an increases distribution of atherosclerosis at areas of increased turbulence such as the origin of the intercostals arteries or branch point of vessels and the prevalence of these early changes increases with age (Stary, 1989;Strong *et al.*, 1999) (Figure 1.2).

Figure 1.2 Prevalence maps of fatty streaks and raised lesions in coronary arteries of young adults.

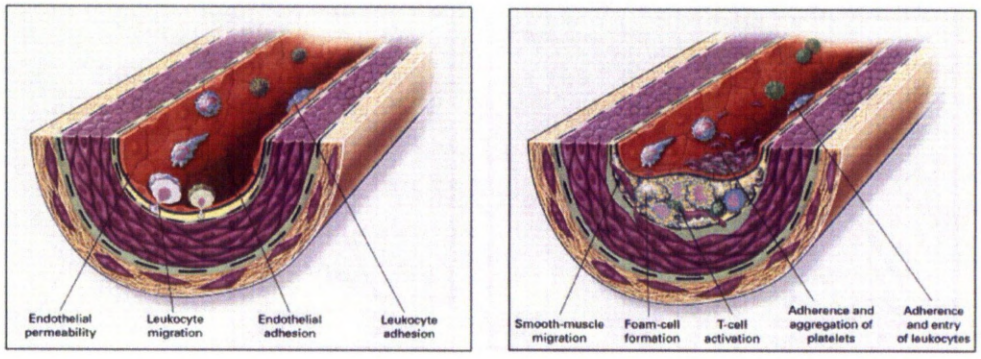


Isopleth maps of fatty streaks (left) and raised lesions (right) demonstrating early detectable changes of atherosclerosis. Strong, J.P. et al. JAMA;281:727-35

One of the earliest detectable change of atherosclerosis is the decrease in bioavailability of nitric oxide and increased expression of cellular adhesion molecules in response to endothelial injury, this often precedes the permeation of lipoprotein

particles through gap junctions between endothelial cells (Simionescu *et al.*, 1986), these coalesce and accumulate in intimal layer by binding to proteoglycans and can be seen with electron microscopy (Camejo *et al.*, 1998). Once in the intima the lipoprotein particles undergo glycation and oxidation and become modified lipoproteins (Witztum and Berliner, 1998). Modified lipoproteins have a tendency to induce cytokines which also cause increased expression of cellular adhesion molecules (CAM) on the surface of the endothelial cells (Figure 1.3). When activated, CAM enable the multi-stage process of monocyte rolling, attachment and diapedesis (Figure 1.3). E-selectin facilitates the capture and rolling of monocytes and, subsequent attachment, facilitated by activated ICAM-1 and VCAM-1, is followed by migration into the intima influenced by signals from other cytokines, particularly monocyte chemo-attractant protein 1(MCP-1) (Luster, 1998;Gu *et al.*, 1998). In the intima, other cytokines including monocyte colony stimulating factor (MCSF) induce monocytes replication and the expression of scavenger receptors enabling the uptake of modified lipoproteins by which monocytes develop into foam cells (Miller *et al.*, 2003). The fatty streak is therefore composed of sparse vascular smooth muscle cells, foam cells which consist of tissue monocytes rich with lipids, and also large numbers of CD4 and CD8 positive T- lymphocytes (Ross, 1993). The net result is a localised thickening of the intima (Fowler, 1980;Klurfeld, 1985). Macrophage foam cells produce further cytokines such as matrix metalloproteinases which stimulate smooth muscle cell migration from the media to the intima, smooth muscle cell proliferation and the production of extra-cellular matrix (Newby, 2008).

Figure 1.3 Early changes of atherosclerosis

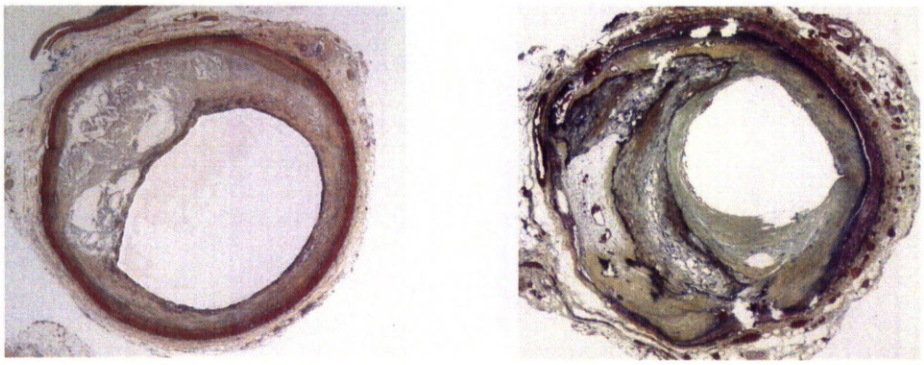


Endothelial dysfunction causes an increase in endothelial permeability to lipoproteins. The response to permeation of lipoproteins is an inflammatory response and recruitment of monocytes, which is mediated by E-selectin, vascular cellular adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1(ICAM-1). The first step is rolling of monocytes whereby the monocytes slow down on the endothelial surface, this is mediated by E-selectin on the endothelial cell and carbohydrate ligand on the monocyte cell surface. The rolling monocyte is susceptible to signals from MCP-1, once stimulated by MCP-1 the monocyte expresses LFA-1, MAC-1 and VLA-4, these enable monocyte to attach onto the endothelial surface and subsequently to migrate into the vessel wall (Left). The monocytes in response to M-CSF transform to macrophages and subsequently engulf oxidised LDL particles in the intima resulting in the formation of the lipid rich foam cell. Foam cells release MMP and free radicals.

T cells are also found in foam cells. Following this smooth muscle cells migrate into the intima from the media mediated by PDGF. Smooth muscle cells which have migrated then divide and result in growth of the plaque. Adapted from Ross NEJM 1999.

Other chemotactic factors such as platelet derived growth factor (PDGF) also mediate smooth muscle cell migration and proliferation (Raines, 2004). As the plaque progresses, layers of foam cells and smooth muscle cells form over the free lipid content, the smooth muscle cells produce large quantities of collagen resulting in the fibro-lipid plaque. The fibrous portion of the plaque consists of a large number of vascular smooth muscle cells (VSMC), infiltrate of inflammatory cells and connective tissue. The core of the plaque is composed of lipid rich material which is the result of dead and dying monocytes, which after absorbing excess cholesterol derived products become lipid laden and die (Bjorkerud and Bjorkerud, 1996;Ross, 1999a), due to toxic effects of this high lipid rich content (Ross, 1993) (Figure 3). The constitution of the plaque can vary considerably and can range from a semi-fluid core which is high in lipid content to very hard fibrous plaques composing of predominantly collagen (Lundberg, 1985;Stary, 1992;Ueda *et al.*, 1997) (Figure 1.4).

Figure 1.4 Histological specimens of atherosclerosis variation in the constitution of plaque



Atherosclerotic plaque with thin fibrous cap (left), fibrocalcific plaque (right). Adapted from Virmani R, et al. Arterioscler Thromb Vasc Biol 2000;20:1262

The clinical presentation of coronary plaques can depend on the composition and characteristics of the lesion. If there is gradual progression of the plaque eventually resulting in a sufficient narrowing of the lumen to affect the flow of blood then the result is angina caused by myocardial ischaemia due to insufficient supply of blood to meet demands of myocardium. The other, and much more serious presentation is with acute myocardial infarction or acute coronary syndrome caused by the rupture of the surface of the plaque and the resulting partial or complete occlusion of the vessel (Shah, 1997;Maseri and Sanna, 1998). This most commonly occurs at the shoulder of the plaque where the cap overlies the edge of the lipid core. This exposes the contents of the plaque, which includes tissue factor and collagen, to react with circulating blood

enabling a powerful pro-thrombotic stimulus which results in the formation of thrombus on the plaque. The thrombus can become incorporated into the body of the lesion causing a sudden progression in the degree of luminal narrowing (Duguid J., 1976).

Atherosclerosis typically affects large to medium sized vessels but does not affect resistance vessels or the microcirculation. However there is an association between microvascular dysfunction and traditional cardiovascular risk factors in the absence of epicardial disease suggesting microvascular dysfunction may be the precursor to the development of obstructive coronary artery disease (Rubinshtein *et al.*, 2010).

1.1.5 The role of cellular adhesion molecules and shear stress in the pathogenesis of atherosclerosis

It has been shown that areas of arterial endothelium at sites that are known to be prone to the development of atherosclerotic lesions, show up-regulation of the inflammatory adhesion molecules VCAM-1 and E-selectin (Bell JP, 2000a). This data was obtained from the carotid bifurcation of the pig, an animal known to develop spontaneous atherosclerosis with both a histological appearance and a spatial distribution very similar to that of the human (Muller *et al.*, 1992).

Very similar changes are seen at the branch points of the intercostal arteries from the aorta, another site known to be prone to the development of atheroma (Bell JP, 2000b). The factor common to these regions is turbulent blood flow, leading to a loss of the

normal laminar shear stress (Asakura and Karino, 1990;Milner *et al.*, 1998). Reduction or loss of laminar flow has been shown to be associated with adhesion molecule expression *in vitro* (Chien *et al.*, 1998). This data suggest that upstream arterial disease, by virtue of producing a reduction in blood flow and hence in shear stress, could result in downstream up-regulation of these adhesion molecules.

Banning *et al.* noted that following balloon carotid angioplasty in the pig model, radio-labelled platelets are deposited distally in the vessel, remote from the area of arterial injury (Banning AP, 1995). The authors were not able to provide an explanation for these results, but it is notable that the region of increased platelet deposition was around the carotid bifurcation, which is precisely the same region in which adhesion molecule up regulation has been shown. A similar phenomenon has been demonstrated to occur *in-vivo* in rat models, in which platelet-leukocyte interaction with the formation of microemboli and plugging of small vessels has been noted (Katayama *et al.*, 2000).

1.2 Inflammation

1.2.1 Inflammation and cardiovascular risk

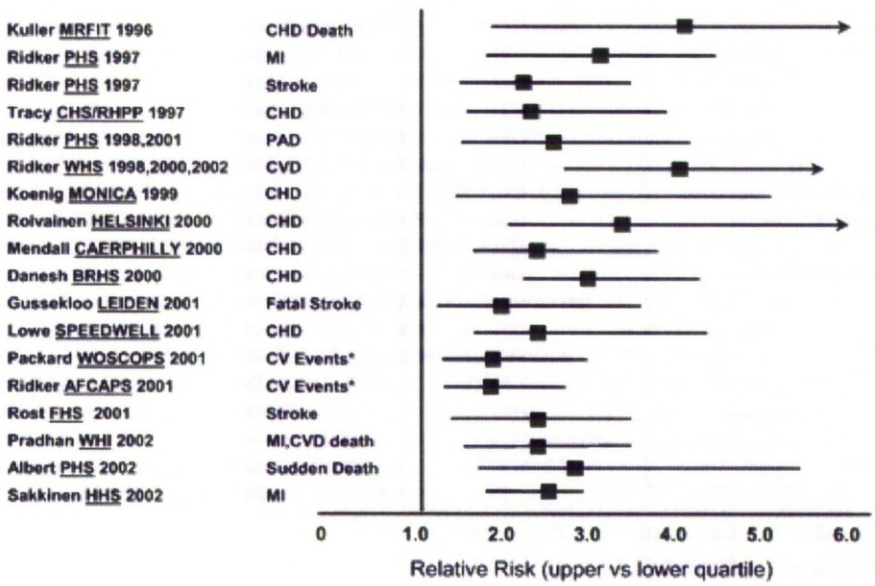
The role of inflammation in the pathophysiology of atherosclerosis has become established in the last two decades. Large scale population based studies have enabled the use of inflammatory biomarkers to assess cardiovascular risk and shown potential for clinical applicability. Several large scale trials have demonstrated the increased

risk of cardiovascular events in apparently healthy individuals with elevated baseline inflammatory markers as discussed below.

A substudy of the Physicians Health Study evaluated the role of elevated C-reactive protein (CRP) in 543 apparently healthy men who subsequently developed cardiovascular disease and compared with age-matched controls without cardiovascular events. They found baseline CRP was higher in the group of patients with cardiovascular events. The relative risk of myocardial infarction and stroke was 2.9 and 1.9 respectively in highest quartile when compared with the lowest quartile of serum CRP levels (Ridker *et al.*, 1997). Similarly, of 14,916 healthy men, 202 had MI in 6 year follow up. When compared with age and smoking matched controls, serum IL-6 levels in the highest quartile had 2.3 fold increased risk of cardiovascular events when compared to the lowest quartile (Ridker *et al.*, 2000b). In addition to Interleukin-6 (IL-6) and CRP , ICAM-1 and serum amyloid A have also been found to be significant predictors of future cardiovascular risk (Ridker *et al.*, 2000a).

Elevated CRP was a greater predictor of future risk of cardiovascular events than serum low density lipoprotein (LDL) levels in a study of 27,939 healthy women followed up for 8 years (RR cardiovascular events was 2.3 and 1.3 for CRP and LDL respectively) (Danesh *et al.*, 2000;Ridker *et al.*, 2002). There are many studies which show a similar increase in risk of cardiovascular events if basal CRP is elevated (Ridker, 2003) (Figure 1.5).

Figure 1.5 Studies comparing baseline CRP levels to risk of cardiovascular events



Association of increased levels of CRP with myocardial infarction, stroke, peripheral vascular disease and death from multiple studies as shown in Figure 1.5 Adapted from (Ridker, 2003).

More recently, the largest ever meta-analysis on the association between inflammation and cardiovascular risk was carried out on over 54 long term studies including approximately 160,000 patients. It compared low CRP vs. 3-fold higher CRP and found the RR of developing CHD was 1.63 in high CRP when adjusted just for age

and sex, this reduced to 1.37 when conventional risk factors were adjusted for and 1.23 if fibrinogen is also adjusted for, with similar reduction in relative risk seen with stroke and vascular mortality (2010). Although this study suggested the significance of CRP on its own may have been overestimated in previous studies due to confounding factors such as traditional cardiovascular risk factors, nevertheless inflammation remains a key risk factor.

It is not surprising conventional risk factors have an additive effect of cardiovascular risk to, since these in themselves promote inflammatory activation due to increased production of angiotensin II, oxidised LDL, pro-inflammatory cytokines produced by adipocytes and advanced glycation end products. These pro inflammatory mediators are found in conditions traditionally associated with coronary artery disease such as hypertension, hyperlipidaemia, diabetes and obesity (Das, 2002).

1.2.2 Modification of cardiovascular risk with statins

With the knowledge that increased inflammation is a major factor in cardiovascular risk, it was interesting to see if modification of inflammation was able to reduce risk. This question was answered by the recent discovery of the role of statins in primary prevention of cardiovascular events. Although statins are drugs designed to reduce circulating LDL and cholesterol, the pleotrophic effects are also known to have a potent anti-inflammatory properties. Statins exert an anti-inflammatory effect due to inhibition of prenylation of intra-cellular transduction proteins involved in cell

signalling. These proteins belong to the Rho and Ras family and perform a multitude of functions, one of which is promotion of inflammatory signals (Liao and Laufs, 2005).

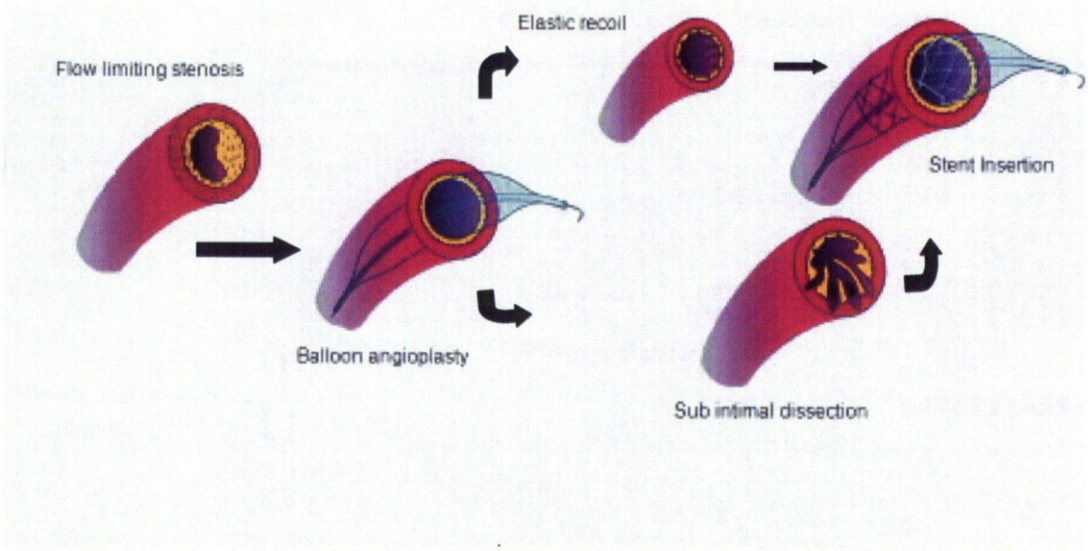
The CARE study assessed the role of pravastatin in over 4000 patients who had a diagnosis of MI within 10 months. The subjects were randomised patients to either 40mg pravastatin or placebo, and all patients had serum cholesterol levels <6.21mmol/L and LDL mean 3.6mmol/L There was a 32% reduction in the incidence of stroke in the pravastatin group, these findings were interesting since at the time the lipid levels used for inclusion into the study were not considered significantly high (Plehn *et al.*, 1999). The AFCAPS TexCAPS study assessed the role of lovastatin in low-moderate risk patients, this study showed benefit even in those patients in whom LDL was normal but with elevated CRP levels (Downs *et al.*, 1998). More recently, the JUPITER study confirmed a large reduction of hospitalisation and revascularisation of 47% compared with placebo in patients with normal LDL <3.4mmol/L and CRP>2mg/L (Ridker *et al.*, 2008). These studies demonstrate cardiovascular protection by the modification of the inflammatory state to some degree, in addition to lowering of LDL cholesterol.

1.3 Percutaneous coronary intervention

1.3.1 Introduction

CHD can be treated by revascularisation or medical therapy to control symptoms and reduce risk of further events. Revascularisation was only possible initially with open heart surgery in the form of CABG until the pioneering of PCI by Andreas Gruntzig in 1977. This involved the passage of a fixed wire balloon catheter into the coronary artery with the lesion and subsequent dilation of the balloon in the coronary stenosis. Initial experience had success rates of up to 60%, but had a high complication rate such as acute vessel occlusion which occurred in up to 8% of patients, most of which required emergency CABG (6%) (Kent *et al.*, 1982;Detre *et al.*, 1988). The other issue with balloon angioplasty was the high rate of restenosis caused by neointimal proliferation of smooth muscle cells, early elastic recoil and arterial remodelling in response to arterial injury from the balloon dilation (Austin *et al.*, 1985).

Figure 1.6 Diagram representing balloon angioplasty and stenting of coronary artery



Representation of balloon angioplasty in coronary artery with possible complications including elastic recoil and dissection of the intima. These initial problems with balloon angioplasty were resolved by placing a stent within the segment of balloon dilated artery. Adapted from Windecker and Meier BMJ;2000.

The incidence of acute vessel closure was addressed by Sigwart *et al.* by the development of the intracoronary stent which acted as a scaffold to keep the vessel open (Sigwart *et al.*, 1987) (Figure 1.6). The issues with restenosis persisted despite stenting with clinically apparent rates of up to 30% (Fischman *et al.*, 1994;Serruys *et al.*, 1994). It was apparent the predominant mechanism of restenosis following stenting was neointimal hyperplasia and thought to be secondary to a prolonged inflammatory reaction (Nakatani *et al.*, 2001). The development of improved stent

technology and drug therapy has substantially reduced the risk of restenosis and improved outcome of PCI whereby approximately 2 million procedures are performed annually worlwide for CHD (Smith, Jr. *et al.*, 2006).

PCI has become increasingly popular, often due to patient preference for a procedure which is minimally invasive and can be performed as a day case in many cardiovascular centers, with return to normal activities in a few days. Current success rates are now well over 95% and over 80,000 PCIs are performed annually in UK. PCI is now four fold more common than CABG as the treatment for CHD and currently half of all PCIs are performed in patients with stable angina (Ludman PF, 2007).

Despite the excellent technological advances and expertise in the field of PCI there remains a small but significant complication rate in the form of the no-reflow phenomenon, poor reflow and periprocedural myocardial infarction or "cardiac enzyme leak", which are both associated with increased mortality and morbidity. It is the underlying mechanisms behind these adverse events which are poorly understood and which form the basis of the hypothesis which is examined in this thesis.

1.3.2 Complications of PCI

1.3.2.1 No-reflow phenomenon

The no-reflow phenomenon denotes the circumstance in which an artery is successfully opened and yet flow into the distal vessels is poor or non-existent, suggesting dysfunction of the resistance vessel bed. A requirement for this phenomenon is temporary occlusion of the artery, which can occur in the setting of

either a myocardial infarction or following PCI. This phenomenon was described in a canine myocardial infarction followed by reperfusion (Kloner *et al.*, 1974a). First observation in humans was made in 1985 whilst performing thallium 201and technetium 99 isotope scans to assess myocardial uptake and perfusion respectively in patients who had undergone acute MI treated with thrombolysis (Schofer *et al.*, 1985). The same phenomenon was described angiographically as abnormally slow anterograde contrast filling in the infarct related artery (Bates *et al.*, 1986).

The incidence of angiographic no-reflow varies from 0.6% to up to 20%, depending on the clinical scenario in which the PCI is performed and the type of procedure (Piana *et al.*, 1994). The incidence is much greater in patients undergoing PCI to treat acute myocardial infarction (primary PCI), saphenous vein graft intervention and rotational atherectomy. There is a four fold increase in mortality (Resnic *et al.*, 2003) and increased risk of MI and heart failure if no-reflow occurs (Ito *et al.*, 1992a;Piana *et al.*, 1994). The most recent data suggests no-reflow is an independent predictor of mortality at 5 years independent of left ventricular infarct size (Ndrepepa *et al.*, 2010). Although angiographic no-reflow is clearly apparent during PCI, there exists a less profound microcirculatory disturbance which has become apparent as the methods of detecting no, or poor reflow have improved with techniques such as myocardial contrast echo and the use of intracoronary Doppler wires. It transpires that the actual incidence of poor or no-reflow, particularly when assessed from the microvessel

perspective is actually much greater than previously thought (Ito *et al.*, 1996b;Kern *et al.*, 1999).

The underlying mechanisms responsible for no-reflow are still unclear although many different factors are believed to be responsible such as distal embolisation of plaque material and thrombus, and the interaction between platelets and leukocytes with the endothelium (Yoon *et al.*, 2006;Tahk *et al.*, 2007). Intravascular plugging of the microcirculation by fibrin and platelets has been shown to play a role and the use of platelet depleted animal models has shown marked reduction in no-reflow (Golino *et al.*, 1987;Seydoux *et al.*, 1993;Michaels *et al.*, 2000). Studies have demonstrated reperfusion in the canine myocardium leads to rapid microvascular accumulation of leukocytes (Engler *et al.*, 1983;Sheridan *et al.*, 1996). The mechanism responsible is thought to be mediated by CD18 dependent leukocyte adhesion (Jerome *et al.*, 1993). Furthermore leukocyte depleted blood in experimental models has shown a reduction in reperfusion injury (Engler *et al.*, 1986a;Engler *et al.*, 1986b;Byrne *et al.*, 1992). Similar results have been obtained in humans undergoing CABG providing further evidence of leukocyte mediation playing a major role in the no-reflow phenomenon (Palatianos *et al.*, 2004).

Inhibition of endothelial adhesion molecules such as ICAM-1 and sP-selectin reduce microvascular damage and no-reflow in a canine model of peripheral circulation (Jerome *et al.*, 1994), and a recent experimental study has shown that dual inhibition

of ICAM-1 and sP-selectin can reduce myocardial infarct size by 65% when administered retrogradely to the post-capillary venules, suggesting that the role of cellular adhesion molecules is also crucial to reperfusion injury, and that this is most marked in the microcirculation (Fukushima *et al.*, 2006). Histologically, cardiac cells subject to reperfusion injury demonstrate damaged endothelium with areas of regional swelling and large intraluminal protrusions which have in some cases appeared to have plugged the capillary lumen (Kloner *et al.*, 1974b). Other structural changes are apparent in experimental models such as extra vascular compression of the capillaries (Gavin *et al.*, 1983;Manciet *et al.*, 1994).

The no-reflow syndrome may respond to treatment with diverse agents including adenosine, the NO donor sodium nitroprusside, the calcium antagonist verapamil and the monoclonal antibody abciximab, an inhibitor of the platelet glycoprotein IIbIIIa receptor (Piana *et al.*, 1994). The glycoprotein IIbIIIa receptor is central to the interaction of activated platelets, both with connective tissue elements within the vessel wall, and also with leukocytes and other platelets. Although the actual mechanisms of both no-reflow itself and the therapeutic agents is unclear, what these agents have in common is the ability to inhibit the aggregation and endothelial attachment of platelets and leukocytes, providing further evidence for their role in the pathogenesis. One of the most popular theories regarding the cause of no-reflow, particularly in the setting of coronary intervention is the suggestion that distal embolisation of plaque material which gets dislodged during the procedure and

thrombus is responsible causing initially a mechanical obstruction (Jaffe *et al.*, 2008). This will be discussed later in the section titled "Distal Embolisation".

1.3.2.2 Periprocedural myocardial infarction

Further evidence that reperfusion may be associated with downstream events is the phenomenon of creatine kinase (CK) "leak" following PCI (Saucedo *et al.*, 2000;Stone *et al.*, 2001). Ever since the technique of balloon angioplasty for the treatment of obstructive coronary artery disease was introduced, complications particularly in the form of myocardial infarction as detected by troponin or CK release, have been encountered (Gruntzig, 1978;Gruntzig *et al.*, 1980). With the improvement in early technology, the rate of acute vessel closure were substantially reduced but the rates of myocardial enzyme release had remained static (Oh *et al.*, 1985). There has been considerable discussion recently on the definition of periprocedural MI and more specifically what level of cardiac enzymes should be used as cut-off to define PMI (Thygesen *et al.*, 2007). The incidence of PMI remains high, affecting up to 40% of subjects depending on the pre-intervention risk profile of the patient, the lesion characteristics and which definition of PMI is used (Herrmann, 2005).

The circulating enzyme CK, a marker of myocardial damage, shows small rises following apparently successful angioplasty and has been shown to be associated with a statistically and clinically significant increase in the subsequent risk of death (Stone *et al.*, 2001;Ioannidis *et al.*, 2003). A recent meta-analysis involving over 15,000

patients shows an increase in mortality in patients with elevation of cardiac specific troponins in patients undergoing elective PCI (Nienhuis *et al.*, 2008). MRI studies have demonstrated that even with mild elevations in the myocardial enzyme creatine kinase-myocardial bound (CKMB) post-PCI correlate with contrast enhanced hyper-enhancement which is synonymous with myonecrosis. Two specific patterns of myonecrosis are seen on MRI scanning, one at the site of stenting, supporting side branch occlusion theory, and one in the target vessel territory supporting downstream microvascular disturbance (Ricciardi *et al.*, 2001;Choi *et al.*, 2004;Selvanayagam *et al.*, 2007).

Side branch occlusion is where there is a side branch of the coronary artery at the site of the intervention in the main vessel, and as a consequence of the angioplasty, the flow in the side branch is compromised causing partial or complete occlusion. Although in some cases the cause of rise in cardiac enzymes may be obvious such as the closure of side branch or vessel dissection, in the majority of cases of cardiac enzyme leak post-PCI, no such trigger is found (Kini *et al.*, 1999). Certain baseline characteristics are strong clinical predictors of periprocedural myocardial infarction such as presence of multivessel disease and systemic atherosclerosis (Kini *et al.*, 1999). Elevated CRP levels pre procedure have also been shown to increase medium term mortality and risk of periprocedural MI suggesting that increased inflammatory state has some effect on the response and risk to the procedure (Chew *et al.*, 2001a).

1.3.2.3 Distal embolisation (DE)

It is well established that PCI causes plaque disruption which can lead to embolization of plaque material or thrombus distally. One of the key theories suggested for the no-reflow phenomenon and peri procedural MI is that of distal embolization following PCI (Henriques *et al.*, 2002;Kotani *et al.*, 2002). The incidence of DE in patients undergoing primary PCI is 15%, assessed by angiography as a distal filling defect with an abrupt closure in one of the peripheral arteries distal to PCI site (Henriques *et al.*, 2002), although the sub-clinical incidence is greater if other methods are used to detect adequate tissue perfusion such as contrast enhanced echo (25%) (Ito *et al.*, 1996a). Histological examination of the contents of distal protection devices have found debris in 75% of patients undergoing PCI (Angelini *et al.*, 2004). The use of distal protection device has been shown to eliminate DE as assessed by Okamura *et al.* They assessed the degree of DE by counting the number of High Intensity Transient Signals (HITS) using a Doppler wire, each HITS is indicative of DE of micro particles. In a primary PCI setting they were able to eliminate HITS with the use of a DPD, suggesting that the use of a DPD is able to prevent DE (Okamura *et al.*, 2005).

However although it is widely accepted that DE occurs in most patients, the evidence the preventing DE improves outcome is equivocal (Gorog *et al.*, 2005). Although a benefit has been shown in the setting of saphenous vein graft intervention, there is clear evidence of no benefit in native coronary arteries in terms of reduction in mortality or reduction in infarct size and left ventricular function (Stone *et al.*,

2005;Cura *et al.*, 2007). The lack of benefit of distal protection devices in the setting of primary PCI in native vessels has raised some important questions regarding the underlying pathophysiology of the poor reflow/ no-reflow phenomena suggesting that the role of DE may have been exaggerated. There is increasing evidence many of the complications seen with PCI may be related to processes occurring at the microcirculatory level.

1.4 Coronary microcirculation

1.4.1 Structure of the coronary microcirculation

The coronary circulation can be divided into three compartments;
arteries, microcirculation, and venous. The micro circulation can be further split into arterioles, capillaries and venules. The epicardial compartment includes vessels ranging from 0.5mm-5mm, the predominant function of these vessels is to provide capacitance and under normal conditions they offer minimal resistance to flow. The small arteries then branch out into pre arterioles, which are 200μm-500μm in diameter and are located on the epicardial surface of the heart. These vessels maintain pressure at the entrance to the arterioles and are able to compensate for changes in flow. These develop into arterioles less than 200μm, which can be further divided into three sub compartments, each characterised by their size, function and predominant regulatory mechanism.

1.4.3 Function of the microcirculation

There is a significant overlap between the mechanisms which regulate the flow of blood into the microcirculation. There is a tendency for each domain of microvessel to have a predominant regulating mechanism, and in the event that one mechanism becomes dysfunctional or is inhibited, then other mechanisms become active to compensate.

The largest of these arterioles are 200-100μm. Kuo *et al* demonstrated endothelial-dependent dilation was most prominent in larger arterioles and least effective in the smallest vessels (Kuo *et al.*, 1995a). In these vessels, an increase in flow rate causes vasodilatation and a reduction in flow causes vasoconstriction (Kuo *et al.*, 1995a). The medium sized microvessels are 40-100μm and the main regulating mechanism is dependant on intraluminal pressure changes in the vessels. Intraluminal pressure is detected by vascular smooth muscle cell stretch receptors which respond to increased intraluminal pressure by causing vasoconstriction. Conversely, a reduction in intraluminal pressure causes compensatory vasodilatation due to smooth muscle relaxation. This pressure sensitive mechanism is known as myogenic control (Kuo *et al.*, 1988a). Endothelial-dependent mechanisms are also present in medium sized microvessels, although the dominant mechanism is myogenic mediation (Kuo *et al.*, 1990b). The smallest arterioles (<30 μm) are regulated by changes in metabolic activity, whereby an increase in metabolic activity leads to vasodilatation (Kuo *et al.*, 1995b). Therefore in the microcirculation, an increase in metabolic activity initially

causes the tiny vessels to dilate, this causes a secondary reduction in pressure upstream in the medium sized vessels causing myogenic dilation leading to increased flow further upstream in the larger arterioles causing endothelial-dependent dilation (Jones *et al.*, 1995). This self- regulating mechanism allows for an integrated sequential activation from the smallest vessels to the largest arterioles in response to increased metabolic demand.

1.4.3 Regulation of the microcirculation

1.4.3.1 Metabolic factors

Broadly speaking, coronary flow is linearly related to myocardial oxygen requirements. An increase in myocardial metabolic activity must be balanced by an increase in the supply of nutrients and oxygen supplied by the blood to the myocardium. This requires an increase in the blood flow to the myocardium since the oxygen carrying capacity of the blood cannot be increased further. The increase in metabolic activity initiates the cascade described above leading to vasodilatation of all the microvessels. Although the precise factors and substances in the pathways involved in this response remain to be elucidated, adenosine and the K_{ATP} channel have been implicated (Berne, 1963;Komaru *et al.*, 1991).

Adenosine was previously thought to be the key mediator of the metabolic response (Berne, 1963), however it is looking increasingly less likely that adenosine is essential for the metabolic response. Kanatsuka *et al.* induced an increase in metabolic demand by pacing canine hearts and compared this with the effects with administering

intravenous adenosine. They found both the effect of pacing and administration of adenosine had similar vasodilatory effects on vessels <200μm in the microcirculation, although pacing also caused dilation of larger vessels up 380μm diameter, suggesting that other factors apart from adenosine are involved in the metabolic response to pacing and factors additional to adenosine appear to play a greater role in larger sized vessels (Kanatsuka *et al.*, 1989). In the same experiment, the smallest vessels demonstrated the greatest degree of vasodilatation, demonstrating an inverse relationship between the size of vessel and response to pacing and adenosine. When the adenosine antagonist, 8-phenyltheophylline was administered concurrently with pacing, microvascular vasodilatation still occurred, and furthermore the levels of adenosine detected interstitially were below vasoactive levels, implying that adenosine is not an essential mediator of the metabolic response (Yada *et al.*, 1999). The addition of adenosine deaminase still allowed for the autoregulatory capacity of the microcirculation to persist, suggesting adenosine is also not critical in autoregulation (Dole *et al.*, 1985). It appears more likely that the role of adenosine in the metabolic response is more of a backup mechanism with other mediators having a greater role. Ishibashi *et al* were able to demonstrate this by studying the exercise induced response of coronary blood flow in chronically instrumented dogs. They found that when nitric oxide (NO) and adenosine pathways are both blocked, there is no reduction in flow however when the K^+_{ATP} channel is blocked, both adenosine and NO act to increase coronary blood flow during exercise (Ishibashi *et al.*, 1998b). Further evidence for the role of adenosine and NO as backup mechanisms was that in the presence of combined

K^+_{ATP} channel blockade and adenosine receptor blockade, NO was able to produce approximately one quarter of the coronary vasodilatation that occurred in response to exercise compared with control. However with complete blockade of all three mediators there was total abolition of the vasodilator response to exercise (Ishibashi *et al.*, 1998a).

These data suggest the K^+_{ATP} channel is critical in regulating the vasodilator response and the roles of adenosine and NO are more of a backup mechanism which comes into action when the K^+_{ATP} channel is suppressed. The K^+_{ATP} channel is present on smooth muscle cells and is activated by acidosis, ischaemia and hypoxia as well as other stimuli such as adenosine and prostacyclin (Komaru *et al.*, 2000). Activation of the K^+_{ATP} channel causes K^+ to leave smooth muscle cells and hyperpolarises the cell membrane leading to inhibition of the voltage dependant calcium channels, thereby reducing intracellular calcium levels and causing smooth relaxation and vasodilatation. Further evidence for the pivotal role of K^+_{ATP} channel as a mediator for dilation of the microcirculation was provided by Sato *et al.* when they administered the K^+_{ATP} channel agonist levkromacalim in a beating canine heart model. Levkromacalim dilates all sizes of microvessels but the most potent effect was in the smallest vessels (Sato *et al.*, 1994). In contrast, blocking this channel with glibenclamide (a K^+_{ATP} channel blocker), caused a reduction in basal flow and vasoconstriction, suggesting the K^+_{ATP} channel is also important in maintaining basal tone (Imamura *et al.*, 1992;Duncker *et al.*, 1995).

Nitric oxide (NO) is also thought to play a role in the metabolic response in the microcirculation since levels of NO increase in response to increased metabolic activity (Bernstein *et al.*, 1996). However, Egashira *et al.* were able to demonstrate blockade of NO had no effect on microcirculatory vasodilatation although epicardial vasodilatation was attenuated (Egashira *et al.*, 1996). It appears NO is not an essential mediator of the metabolic response.

1.4.3.2 Myogenic factors

The maintenance of intra-luminal pressure within fine limits in the microcirculation is necessary to ensure adequate transport of substances across the vascular lumen to the tissues. In the face of elevated intra-luminal pressure there is a risk of tissue damage due to oedema. In the medium sized arterioles the main mechanism involved in regulation of the microvascular circulation is alteration of the smooth muscle contractile tone in response to changes in intraluminal pressure. Kuo *et al.* demonstrated that the underlying mechanism behind the myogenic response in isolated microvessels is independent of endothelial function (Kuo *et al.*, 1990a). It is thought that rise in intraluminal pressure activates a stretch receptor on the surface of the smooth muscle cell causing cation influx and subsequent depolarisation of the smooth muscle cell, activating the voltage gated calcium channel (L-type) and allowing for calcium influx into the cell (Nelson *et al.*, 1990;Davis *et al.*, 1992). It is well established that intermediate sized arterioles (50-80μm) have the greatest degree of myogenic tone (Kuo *et al.*, 1995c). A possible explanation may be the distribution of

L-type calcium channels in the microcirculation. Bowles *et al.* were able to show an inverse relationship between vessel diameter and concentration of L-type calcium channels (Bowles *et al.*, 1997). Apart from the role of the L-type calcium channel, the stretch sensitive Phospholipase C (PLC) may also implicated in the myogenic response (Park *et al.*, 2003).

The exact mechanisms of mechano-transduction initiating and sustaining myogenic tone remain unclear. The mechanical stimulation of the smooth muscle cells appears to activate G proteins coupled to PLC, increasing PLC activity leading to elevated diacylglycerol levels, further activating protein kinase C (PKC). PKC increases the activity of non-selective cation channels, depolarizing the smooth muscle membrane potential and enhancing calcium entry through voltage-dependent calcium channels. Calphostin C (a PKC inhibitor) has been shown to inhibit the myogenic response and PKC agonist has enhanced the myogenic response (Miller, Jr. *et al.*, 1997;Davis and Hill, 1999). In addition, there is evidence of transmural heterogeneity: studies suggest myogenic responses are reduced in the subendocardium compared with the subepicardium, this could also may explain why subendocardium is more prone to ischaemic injury particularly given the increased physical stresses in the subendocardium during the cardiac cycle (Kuo *et al.*, 1988b). The K^+_{ATP} channel, in addition to its central role in the metabolic regulation, has also been implicated as a mediator for the myogenic response. Glibenclamide, a K^+_{ATP} channel inhibitor, has been shown to inhibit the myogenic response (Komaru *et al.*, 1991). In summary, the

myogenic response is endothelium-independent and regulated by intracellular calcium levels mediated via the voltage gated calcium channels and L-type calcium channels in the cell membrane of the smooth muscle under the influence of PKC and PLC.

1.4.3.3 Endothelial factors

An endothelial-dependent mechanism has been identified as been responsible for flow mediated dilation (FMD) in coronary arterioles. Three major mediators are thought to be responsible; NO, endothelial derived hyperpolarising factor (EDHF), and prostacyclin. The production and release of these mediators are triggered by shear stress detected on the endothelial cell surface receptor. In addition, the endothelial cell layer is responsible for the production of potent vasoconstrictors and the maintenance of endothelial tone is dependent on a fine balance between endothelial derived vasoconstrictors and vasodilators.

1.4.3.4 Vasodilators

The first endothelial derived vasodilator to be discovered was prostacyclin (Moncada *et al.*, 1976). Prostacyclin is synthesized in endothelial and smooth muscle cells from arachidonic acid by cyclooxygenase and binds to smooth muscle receptors stimulating an intracellular increase in cAMP via adenyl cyclase subsequently causing increase in cAMP and smooth muscle relaxation (Koller *et al.*, 1993).

NO was discovered as the main component of endothelial derived relaxing factor by Palmer *et al* (Palmer *et al.*, 1988a). NO is synthesized from L-arginine by the action eNOS, which is a calcium/ calmodulin dependent enzyme. It is a key mediator in the larger arterioles and production is mediated by signal transduction by receptor bound G proteins sensitive to shear stress, which cause an increase in intracellular calcium and eNOS activation. NO acts on soluble guanylate cyclase causing smooth muscle relaxation by inhibiting the transport of calcium into the cell, so causing a reduction in intracellular calcium levels and hence vasodilatation (Ignarro *et al.*, 1999). In addition to shear stress, NO release is also stimulated by thrombin, serotonin adenosine diphosphate, histamine and bradykinin (Moncada *et al.*, 1991). In addition to these vasorelaxant properties, it has subsequently become apparent that NO has anti-inflammatory properties which seem to protect the endothelium from adhesion and infiltration from leukocytes by inhibition of adhesion molecules and cytokines (Armstrong, 2001). All of these actions are important in the development of the atherosclerotic plaque and it is no surprise that reduced production of NO is associated with both the development and progression of atherosclerosis (Lee and Libby, 1997).

More recently there has been the discovery of an additional endothelial derived relaxing factor which acts independently of NO and cycloxygenase pathways known as endothelial derived hyperpolarising factor (EDHF). Although the specific compound is as yet unidentified and there is much speculation to its identity. The discovery came about when both NO and cycloxygenase pathway were inhibited and

an endothelial agonist administered, the surprising finding of a sparing of the vasodilator response suggested another endothelial derived factor was active (Chen *et al.*, 1988;Feletou and Vanhoutte, 1988). If concurrent K^+ channel blockers are given, vasodilatation is abolished thereby suggesting vasodilation is dependent on some form of hyperpolarising factor. EDHF seems to be released in response to shear stress and causes hyperpolarisation of vascular smooth muscle cells by opening calcium activated K^+ channels (Miura *et al.*, 2001).

1.4.3.5 Vasoconstrictors

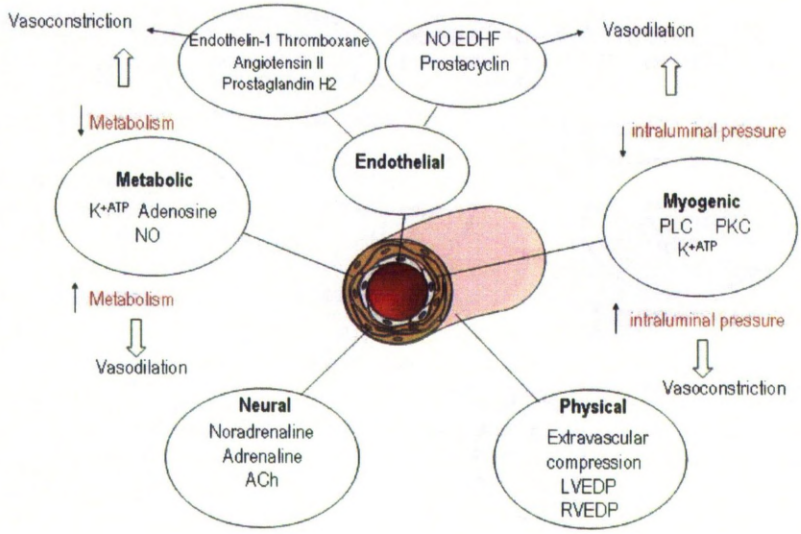
The endothelium produces a wide variety of vasoconstrictors including thromboxane A2 prostaglandin angiotensin II and endothelin-1 (ET-1) (Verma and Anderson, 2002). ET-1 is produced from a large pre pro-endothelin polypeptide, endothelial activation causes proteolytic breakdown of pre-ET into active ET-1. ET-1 is the most potent vasoconstrictor known, which also stimulates leukocyte adhesion, recruitment and smooth muscle cell migration (Yanagisawa *et al.*, 1988). The maintenance of arterial tone and healthy endothelium is dependent on a fine balance between endothelial derived vasoconstrictors and vasodilators, and imbalance leads to endothelial dysfunction.

1.4.3.6 Other factors

As well as the metabolic, myogenic and endothelial mechanisms discussed earlier, there are many other factors which all play a role in the control of the

microcirculation, such as the autonomic nervous system and extravascular physical forces from the effect of the beating heart (Figure 1.7). Subendocardial perfusion in particular is reduced by physical forces related to the beating heart, partially explaining why the subendocardium is most prone to ischaemia and infarction.

Figure 1.7 Schematic diagram representing different mechanisms responsible for the regulation of myocardial microvascular resistance



Several factors are involved regulating the tone of the microcirculation. Endothelial dependent regulation is mediated by the release of vasoactive mediators from endothelial cells in response to stimuli such as shear stress and platelet derived mediators. This is the dominant mechanism in larger arterioles. Myogenic regulation utilises stretch receptors in the smooth muscle cells of the microvessels. The activation of smooth muscle membrane bound ionic channels respond to changes in intraluminal

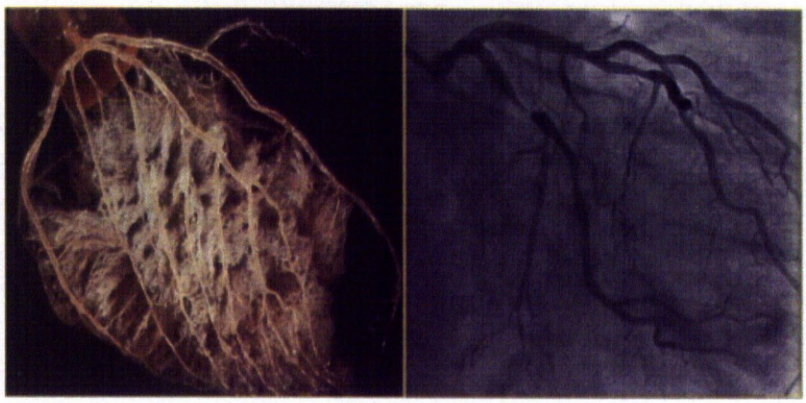
pressure and is the dominant mechanism in medium sized vessels. Metabolic factors such as change in PCO_2, PH and adenosine contribute to vasodilation in response to an increase in metabolic activity, and this is most prominent in the smallest microvessels. In addition neurally mediated factors and extravascular mechanical forces are involved in the regulation of myocardial microvascular resistance (ACh, Acetylcholine; K^{+ATP}, ATP sensitive K+ channel; EDHF, endothelial derived hyperpolarising factor; LVEDP, left ventricular end diastolic pressure; NO, nitric oxide; PKC, protein kinase C; PLC, phospholipase C; PCO_2, partial pressure of carbon dioxide; RVEDP, right ventricular end diastolic pressure).

1.4.4 The clinical significance of the myocardial microcirculation

There is a close relationship between myocardial blood flow and oxygen tension in venous blood. Blood flow increases proportionally to a reduction in coronary sinus oxygenation, suggesting that coronary resistance vessel tone is determined by the equilibrium between oxygen supply and demand (Tune *et al.*, 2004). The control of myocardial blood flow lies in the ability of the myocardial microvasculature to redistribute resistance. There are approximately forty five millilitres of blood in the coronary circulation at any one time. This blood volume is split equally between the arteries, veins and microcirculation with 90% of the blood in the microcirculation being in the capillaries (Kassab *et al.*, 1994). During states of increased demand such as exercise, there is a large shift in distribution of blood within the coronary circulation secondary to arteriolar dilatation resulting in a much greater volume of blood residing

in the microvascular compartment (Figure 1.8). The earlier animal studies had found resistance is heterogeneously distributed throughout the coronary circulation with approximately 50% of total myocardial resistance accounted for by vessels smaller than 100μm, and a further 25% in vessels greater 100μm-200μm, the remaining resistance in vessels greater than 200μm, demonstrating small arterioles are the key regulators of myocardial microcirculation (Chilian *et al.*, 1986). More recent echo studies in humans have showed similar findings (Jayaweera *et al.*, 1999). It is this ability to modify the resistance dynamically which gives the myocardium the ability to increase flow dramatically when the need arises. Hence by measuring microvascular resistance, important information can be obtained regarding the myocardial microcirculation.

Figure 1.8 Coronary microcirculation



Post mortem cast of coronary circulation(left), demonstrating the complex intricate nature of the coronary microcirculation which cannot be visualised with in vivo techniques which are effective at visualising epicardial disease such as coronary

angiography.(right) (Reprinted by permission of the Society of Nuclear Medicine from: Camici PG and Rimoldi OE. The Clinical Value of Myocardial Blood Flow Measurement. J Nucl Med. 2010; 50(7): 1076-1087)

Microvascular injury following reperfusion, in patients with acute myocardial infarction, is a predictor of adverse outcome (Gibson *et al.*, 2000;Yamamuro *et al.*, 2002). In patients with hypertrophic cardiomyopathy, the degree of microvascular dysfunction is strongly associated with poor prognosis (Cecchi *et al.*, 2003). A similar adverse association is seen with microvascular dysfunction and outcome in the recipients of cardiac transplants (Tona *et al.*, 2006). The importance of microvessel patency is now recognised as one of the major components in successful reperfusion and despite apparently normal epicardial flow, microvascular flow can be impaired and is strongly associated with poor prognosis (Ito *et al.*, 2004). Since measurement of microvascular integrity is complex it has often been overlooked but now the use of techniques which can assess the microcirculation with ease during PCI could provide valuable prognostic information (Fearon *et al.*, 2008). Assessment of the microcirculation may also provide a better understanding of the poor/no-reflow phenomenon, alluded to previously.

1.4.5 Assessment of microvascular function

1.4.5.1 Introduction

As noted above, the coronary microcirculation regulates blood flow by responding to increased cardiac metabolic demands. Despite this important role, study of the microcirculation has been neglected for many years. It has been difficult to evaluate the function of this compartment, and doing so conflicts with the current clinical practice of many cardiologists, who are more familiar with dealing with the disease processes that affect the large epicardial arteries. The clinical importance of microvascular function is emerging because of improving techniques which allow for the objective assessment whilst in the catheter laboratory. In addition, there is a growing body of evidence to suggest that the microvascular compartment may show early changes in patients who are at risk of coronary artery disease (Halcox *et al.*, 2002;Rubinshtein *et al.*, 2010). It seems highly likely that the microcirculation is responsible for the poor response to revascularisation in certain patients, potentially in the form of the no-reflow phenomenon and periprocedural myocardial infarction. Pathological microvascular changes could explain the significant midterm morbidity and mortality associated with these complications.

1.4.5.2 Assessment

In humans, assessment of the coronary microcirculation is based on a functional rather than an anatomical basis due to difficulties in experimenting on human microcirculation in vivo. Selective coronary angiography only demonstrates the

conduit vessels and the resistance vessels are too small to be catheterised selectively (Figure 1.8). Hence indirect methods are employed, using indices which reflect functional status, such as coronary or myocardial blood flow. Coronary and myocardial flow can be assessed by various methods, but coronary flow only provides partial information on the state of the microcirculation. Since true myocardial microcirculatory resistance is defined as the ratio between myocardial perfusion pressure and absolute myocardial blood flow, measurement of intracoronary pressure is essential (Figure 1.9).

Figure 1.9 Definition of true myocardial resistance

$$\textit{Myocardial resistance} = \frac{\textit{myocardial perfusion pressure}}{\textit{absolute myocardial blood flow}}$$

True myocardial resistance can be calculated from myocardial perfusion pressure and absolute myocardial flow as shown in figure 1.9. Although myocardial perfusion pressure is relatively simple to assess, the same cannot be said for absolute myocardial flow.

1.4.5.3 Methods of assessment

Many techniques allowing for assessment of myocardial or coronary blood flow are used in the research setting. These consist of invasive and non-invasive methods and vary in the degree of complexity of performing the test and interpretation of results.

By utilising simple coronary angiography measurements such as Thrombolysis In Myocardial Infarction (TIMI) flow grade (TFG)(1985), TIMI frame count (TFC) (Gibson *et al.*, 1996) and myocardial perfusion grade (MPG) (Hof *et al.*, 1998), microvascular function can be assessed. Although these techniques are relatively simple to perform and interpret they do have limitations. MPG and TIMI flow grade are subjective measures with significant degrees of intra-observer variability (Gibson *et al.*, 2000), and TFC lacks correlation to true flow (Chugh *et al.*, 2004). Coronary sinus thermodilution can be used to measure global myocardial blood flow but is unable to provide regional assessment (Ganz *et al.*, 1971). The advent of intracoronary wires such as the Doppler wire and RADI™ pressure wire have revolutionised the assessment of microvascular function and are valuable tools in intracoronary physiology.

1.4.5.3.1 Doppler wire

The Doppler wire has a tiny piezoelectric crystal embedded near the tip of the wire, the crystal is able to transmit and receive ultrasound frequency waves (Doucette *et al.*, 1992). It is possible to detect the movement of red blood cells in the vessel which causes a shift in ultrasound frequency. The frequency of the reflected signal is linearly related to blood flow, hence the magnitude of shift in velocity is proportional to blood velocity and hence flow, as long as vessel diameter is held constant (Franklin *et al.*, 1961). Currently used wires are 0.014" in diameter and are able to detect both blood flow velocity and intracoronary pressure simultaneously (Siebes *et al.*, 2004).

There are certain considerations that must be taken into account when using the Doppler wire. The degree of Doppler shift is dependent on the angle of difference between the piezoelectric crystal and the direction of blood flow. The quality of the signal and the value of the peak velocity detected are dependent on consistent and careful positioning of the wire. The comparability of two measurements made at different times may differ if the wire position changes, since small shifts in the angle of the tip of the wire produce significant changes in the measurements and it can be difficult to get reliable signal in up to 30% of patients (Piek *et al.*, 2000;Barbato *et al.*, 2004a). The position of the Doppler sensor in relation to the lesion and any side branches is also important. If the sensor is placed too close to a lesion or a side branch, velocity measurements will be affected by increased turbulence due to flow separation (Doucette *et al.*, 1992).

1.4.5.3.2 The temperature sensitive pressure wire

The temperature sensitive pressure wire has a high fidelity sensor embedded in the distal portion 3cms from the tip, allowing for simultaneous measurement of temperature and pressure. The shaft of the wire acts as the proximal thermister by monitoring changes in electrical resistance which are temperature dependent.
If the wire is inserted into a coronary artery, a bolus of saline can be injected into the vessel, then according to the indicator dilution theory, the mean transit time (T_{mn}) of the intracoronary injectate to travel from the site of injection to the distal sensor is

inversely related to flow (De Bruyne *et al.*, 2001). This technique is discussed in depth in Chapter 2.

Apart from invasive assessment there are many non-invasive techniques which have been developed which estimate myocardial and coronary flow, including myocardial contrast echo (Keller *et al.*, 1989), PET (Araujo *et al.*, 1991) and myocardial perfusion MRI (Manning *et al.*, 1991). The advantage of these techniques over invasive measurements is the ability to perform serial measurements in the same patient without instrumentation. However for patients undergoing coronary intervention, intracoronary wires which either use the Doppler or thermodilution method are more suited. Firstly since no additional instrumentation of the coronary arteries is required because these wires also serve as angioplasty guide wires, and secondly acute physiological response to PCI can be assessed.

1.5 Summary

In summary, we have found evidence that inflammatory processes are involved in both early and late stages of the development of atherosclerotic lesions, with a sound mechanistic basis. Systemic markers of inflammation are raised at the time of acute ischaemic events, and the extent of the rise has prognostic significance. What remains unexplained, however, is the fact that revascularisation treatment is itself associated with a rise in inflammatory markers which is independent of the rise in troponin and prognostically significant (Saleh *et al.*, 2005). We have described evidence suggesting

that downstream events are associated with PCI, and that these events are associated with myocardial injury (Abdelmeguid *et al.*, 1996). However, the mechanisms underlying these observations are not known.

We suggest a unifying hypothesis that attempts to link mechanistically all of the observations described above, with a view to possible clinical therapy. We therefore suggest that when flow is arrested, for instance by balloon inflation during PCI, leukocytes bearing the receptors for these adhesion molecules would have increased opportunity to adhere to the activated endothelium. This would then lead to leukocyte activation, which in turn would result in direct endothelial damage and expression of cell surface receptors for platelets by the leukocytes themselves. The resulting endothelial injury would lead to reduced flow in the vessel and distal microcirculation. We suggest this would be more likely to occur in patients who already have increased levels of baseline inflammation and hence endothelial activation and an increased propensity to microvascular dysfunction.

1.6 Original hypotheses

1. PCI in stable coronary disease causes myocardial microcirculatory dysfunction.

2. Increased levels of generalised baseline inflammation and endothelial activation are associated with myocardial microcirculatory dysfunction following PCI.

3. The extent of inflammatory activation to PCI is associated with the extent of myocardial microcirculatory dysfunction.

Chapter 2 Methods

2.1.1 Ethical approval

The study protocol was approved by local institutional review board and ethical approval has been granted by the Liverpool Research Ethics Committee and Administration of Radioactive Substances Advisory Committee.

2.1.2 Project design and recruitment of subjects

Subjects with a diagnosis of IHD scheduled to undergo single vessel PCI to either the left anterior descending (LAD) or circumflex branches of the left coronary artery were prospectively recruited. Patients were selected from referrals received to the Liverpool Heart and Chest Hospital pooled referrals list for elective PCI. If the coronary angiogram confirmed significant stenosis (defined as greater than 70% stenosis) as assessed by the referrer and one member of the research team, and provided no exclusion criteria were apparent from the referral letter, then the patient was approached by telephone call and asked permission to send a written patient information leaflet. If the patient agreed to be considered for inclusion into the study then a full discussion regarding the details of the study took place. If deemed suitable to include in the study and if the patient agreed then written informed consent was obtained. Subjects were excluded if any factors were present which complicate the measurement of microvascular function or have an effect on the inflammatory

response to PCI, these are discussed in chapter 3. Each subject acts as their own control since we are comparing the same patient before and after intervention.

Subjects attended the pre-assessment clinic in the cardiology outpatients department on the ground floor of the Liverpool Heart and Chest and Hospital where clinical evaluation was carried out including full medical history, drug history, physical examination 12 lead ECG and baseline bloods for haemoglobin, neutrophil count, LDL, HDL. Angina severity was assessed using the Canadian Cardiovascular Society (CCS) classification. Height and weight were measured and body mass index (BMI) calculated.

2.2 Assessment of microvascular function

In humans, assessment of the coronary microcirculation is based on a functional rather than an anatomical basis due to difficulties in experimenting on human microcirculation *in-vivo*. Selective coronary angiography only demonstrates the conduit vessels and the resistance vessels are too small to be catheterised selectively (Figure 1.8), hence indirect methods are employed, using indices which reflect functional status, such as coronary or myocardial blood flow.

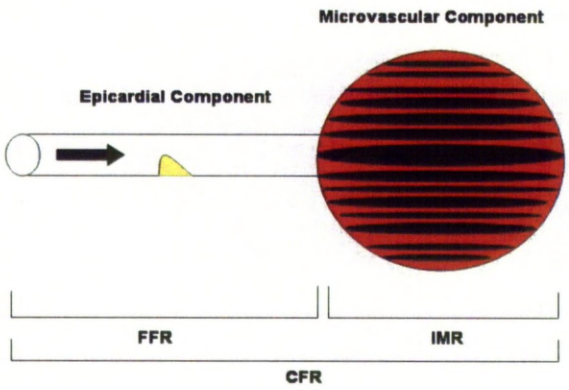
Coronary and myocardial flow can be assessed by various methods, but flow only provides partial information on the state of the microcirculation. Since true myocardial microcirculatory resistance is defined as the ratio between myocardial perfusion

pressure and absolute myocardial blood flow (Figure 1.9), measurement of intracoronary pressure is essential. We opted for the thermodilution method to assess coronary flow due to the limitations of the Doppler wire method especially since we wish to perform repeated measurements in different vessels and small changes in the angle of the sensor can produce significant changes in velocity and hence flow measurements.

2.2.1 Justification of method used to calculation of MR

With the use of the different methods described above, certain indices of microvascular function can be calculated. The most widely used is the coronary flow reserve (CFR).

Figure 2.1 Two compartment model of the coronary circulation



Fractional flow reserve (FFR) assesses epicardial component, index of microcirculatory resistance (IMR) assesses the microvascular component and coronary flow reserve (CFR) assesses both epicardial and microvascular components combined as shown in Figure 2.1.

2.2.1.1 Coronary flow reserve (CFR)

The CFR is the maximal possible increase in blood flow in a coronary artery compared to the resting state (Gould *et al.*, 1974). This is assessed by measuring the ability of the microcirculation to respond to hyperaemic stimuli. The hyperaemic stimulus causes profound microcirculatory vasodilatation thereby increasing flow through the coronary artery and into the myocardial territory supplied by the vessel. CFR can be calculated with the formula shown below.

$$CFR = \frac{\text{coronary flow at maximal hyperaemia}}{\text{coronary flow at rest}}$$

Gould *et al* demonstrated a reduction in CFR once epicardial stenosis was greater than 30%. As the degree of epicardial stenosis increases, the microcirculation dilates to maintain flow, although resting flow remains unaffected until the stenosis was greater than 80% (Gould *et al.*, 1974). Thus when hyperaemia is induced, the degree of microvascular dilatory reserve is reduced due to a pre-vasodilated microcirculation and hence CFR is less. The relationship between degree of epicardial stenosis and CFR displays a large degree of scatter largely due to the poor reliability of the coronary angiogram as a marker of physiological severity (White *et al.*, 1984). Given that the value of CFR is dependent on blood flow at rest, a significant amount of variability of CFR in the same individual may be present. CFR is also affected by haemodynamic factors such as blood pressure and pulse rate (Gould *et al.*, 1990). An increase in heart rate by 15 beats per minute can reduce CFR by as much as 15% due to an increase in resting flow (McGinn *et al.*, 1990). Although initially used as a marker of epicardial disease, CFR also detects microvascular dysfunction, since CFR assesses both the microcirculatory and epicardial components (Figure 2.1). In cases of normal epicardial vessels or no flow limiting epicardial disease, CFR can be used to measure microvascular function independently, since abnormal CFR would suggest pure microvascular dysfunction. In patients with established CAD the significance of an abnormal CFR is less clear and the degree of resistance offered by the epicardial and microvascular compartments cannot be distinguished without further investigation (Meuwissen *et al.*, 2001a).

In summary CFR is particularly useful for microcirculatory assessment if epicardial vessels are normal. Although CFR has been used as detector of the physiological significance of epicardial stenosis, in practical terms there are better physiological measurements which are less dependent on microcirculatory factors and haemodynamic factors such as fractional flow reserve (FFR) (De Bruyne *et al.*, 1996).

In our study, all patients have significant epicardial disease pre-PCI, and this will have an adverse effect on CFR, although post-PCI the effect of the epicardial disease should no longer be present but we know from previous studies that CFR can be reduced post-PCI due to increases in basal flow (Kern *et al.*, 1999). Hence CFR will also be an underestimation of true MR post-PCI. Ideally we need a measure of MR which will be independent of epicardial stenosis and independent of baseline haemodynamic factors.

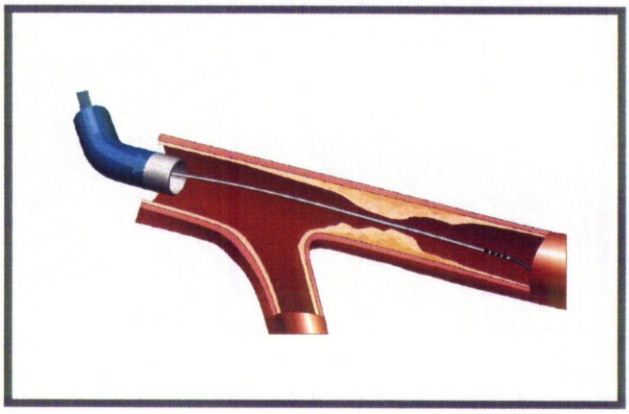
2.2.1.2 Thermodilution principle to measure coronary flow reserve

As mentioned earlier the RADI™ pressure wire can be used to calculate coronary flow by utilising the thermodilution method. The indicator dilution principle dictates that flow can be calculated in a circuit if an injection of a known quantity of indicator is introduced into the system and by measuring the concentration of the indicator down stream, flow can be calculated (Stewart, 1921). This principle was further developed to measure temperature of the indicator rather than concentration by measuring change in temperature downstream from the site of injection, this could then be used to calculate

an estimate of flow. This technique was described by Fegler to assess cardiac output in a canine model (FEGLER, 1954).

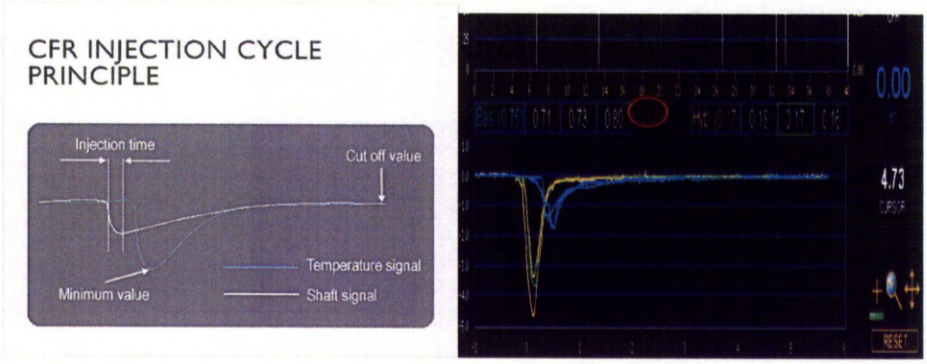
With the development of intracoronary wires (Figure 2.2) such as the RADI™ wire, it is possible to measure temperature and pressure simultaneously within the coronary artery and hence estimate flow and pressure simultaneously (De *et al.*, 2001). By utilising the thermodilution method, the mean transit time, defined as the time it takes for the bolus of injectate to travel from site of injection (the coronary guide catheter), to the sensor (in the coronary artery) in the distal third of the vessel, can be measured.

Figure 2.2 Diagram representing the intracoronary pressure wire.



The wire is directed down the coronary artery through the coronary guide catheter (blue) and beyond the lesion. The sensor located near the tip of the wire is able to measure changes in intracoronary pressure and temperature; hence microvascular resistance can be calculated.

Figure 2.3 Thermodilution curves obtained with RADI™ wire.



The blue line represents the temperature tracing from the distal sensor, and the white line from the shaft which acts as the proximal sensor. The proximal sensor curve is used to detect the start of the injection (left). Recorded data from RADI analyser (right) only shows distal sensor data, there are two sets of data representative of mean Tmn at rest (yellow line) and at hyperaemia (blue line), the shaft signal is not shown on the live data (right), but is used by the analyser to calculate the start time of the injection. The analyser calculates the T_{mn} (red circle). The x-axis represents time in seconds and the y-axis represents temperature (Right)

Figure 2.4 Calculation of mean transit time of injectate

$$T_{mn} = \frac{\int_0^\infty t \cdot c(t)dt}{\int_0^\infty c(t)dt}$$

The mean transit time of injectate is calculated with the following equation as shown in Figure 2.4. Tmn = mean transit time, c(t)= distal dilution curve.

According to the indicator dilution principle flow can be calculated if we know volume and mean transit time of a bolus of injectate.

$$Flow \propto \frac{Volume}{Tmn}$$

When using this method certain assumptions are made according to the indicator dilution principle, such as the volume between the injecting sites and the monitoring site must remain constant both at rest and during hyperaemia. Therefore the position of the sensor must remain unchanged between measurements. Secondly the diameter of the epicardial vessel must remain unchanged since with the induction of hyperaemia vasodilation occurs at epicardial level due to endothelial-dependent flow mediated dilation. To counter the effect of flow mediated dilation an epicardial vasodilator such as glyceryl trinitrate (GTN) can be administered. Finally there must be adequate mixing of the injectate with blood for the thermodilution principle to be accurate, by placing the sensor more distally in the vessel prevents inadequate mixing.

CFR can easily be calculated with the thermodilution technique by dividing the coronary follow during hyperaemia by flow at rest (Figure 2.5). This method correlates closely with the Doppler method in experimental and human models model with CFR (De *et al.*, 2001;Pijls *et al.*, 2002a). We have previously discussed the

limitations of CFR as a measure of microvascular function, thus another method of measuring MR which is independent of these factors is preferable for our study.

Figure 2.5 Calculation of CFR thermodilution

$$CFRtherm = \frac{\left[\frac{1}{Tmn(hyper)}\right]}{\left[\frac{1}{Tmn(rest)}\right]} = \frac{Tmn(rest)}{Tmn(hyper)}$$

2.2.1.3 Doppler based assessment of microvascular resistance (MR_v)

Using the Doppler wire a simpler measurement of MR can be made called MR_v. This method calculates intracoronary flow by measuring the velocity of blood in the vessel, which is proportional to flow. Distal pressure can be measured with the same wire and both values into the equation below (Figure 2.6). The volume in the coronary artery is assumed to be constant. The administration of intracoronary nitrates minimises changes in intravascular volume.

Figure 2.6 Calculation of myocardial microvascular resistance using intracoronary Doppler

$$MRv\ hyp = \frac{pressure}{flow(v)} \quad during\ hyperaemia$$

$$MRv\ b = \frac{pressure}{velocity} \quad during\ basal\ conditions$$

MR_v was described by Meuwissen *et al.*, (Meuwissen *et al.*, 2001b). They investigated 150 intermediate epicardial lesions in 126 patients and measured FFR and CFR. In this study, cut of values of less than 0.75 and 2 were used respectively as abnormal. There was discordance in 25% of measurements and when MR_v was measured, they were able to show that in patients who had normal FFR but low CFR the MR_v was greater than patients who had low FFR but normal CFR, although MR_v was greatest in the group with low FFR and low CFR, who by definition had haemodynamically significant lesions by both methods. Thus the importance of measuring MR_v particularly where the FFR and CFR does not match was, demonstrated and they concluded that abnormalities in MR could account for variability in haemodynamic measurements. The effect of microvascular resistance was thus found to be an important factor in a significant proportion of subjects.

2.2.1.4 Index of microcirculatory resistance (IMR)

IMR is a novel measure of microcirculatory resistance in the coronary microvasculature described by Fearon *et al.* (Fearon *et al.*, 2003). Coronary flow can be assessed as described above using the thermodilution principle and the intraluminal pressure in the coronary artery distal to the lesion can be measured with the pressure wire. When both of these measurements are recorded during maximal hyperaemia, microvascular resistance can be calculated using the following equation (Figure 2.7).

Figure 2.7 Calculation of IMR

$$Myocardial\ Microvascular\ Resistance = \frac{coronary\ pressure}{flow}$$

$$= \frac{Pd}{flow}$$

$$\approx \frac{Pd}{\left(\frac{V}{Tmn}\right)}$$

V is assumed to be constant since intracoronary nitrates are given to achieve maximal epicardial dilatation.

$$Myocardial\ Microvascular\ Resistance \approx P_d \times T_{mn}$$

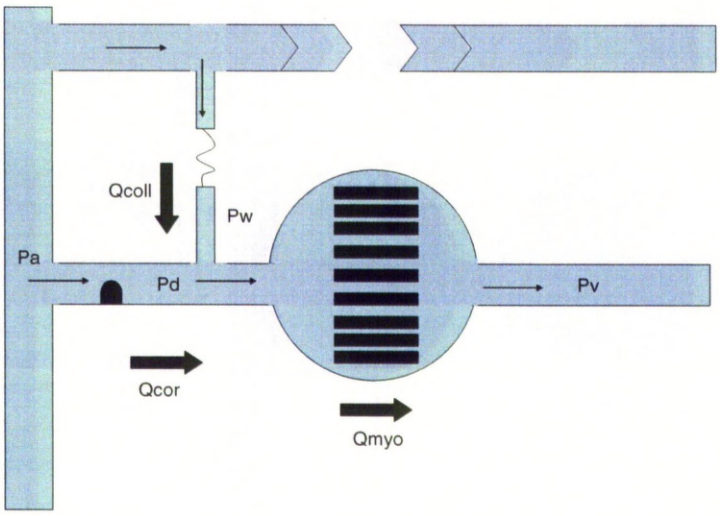
IMR was first measured in an experimental swine model to detect microvascular dysfunction. When compared with total myocardial resistance calculated from ultrasonic flow probe around the proximal LAD there was a good correlation between IMR and total myocardial resistance (TMR) (Fearon *et al.*, 2003). A physiological representative model compared IMR vs. TMR in different physiological conditions found close correlation between true MR as induced by distal resistance clamp, and IMR (Aarnoudse *et al.*, 2004b). The advantage of minimal MR over basal measurements was the variability of resting tone and baseline factors were taken away, since the microcirculation is near maximally dilated. In addition this method was no longer constrained by haemodynamic factors which are variable in the resting state. This notion was confirmed in a human model where IMR was found to be independent

of changes in pulse rate, blood pressure and left ventricular contractility (Ng *et al.*, 2006).

2.2.1.4.1 Effect of collateral flow on IMR

One of the drawbacks of using coronary flow to assess myocardial resistance is the assumption coronary flow equals myocardial flow. Often in patients undergoing measurements, significant epicardial stenosis are present, therefore coronary flow may not equal myocardial flow if there is significant collateralisation (Figure 2.8). Thus the concept of IMR was developed one step further by measuring the collateral component of myocardial flow, and it was suggested coronary wedge pressure should be measured, this could then be used to correct for the contribution of collateral flow (Fearon *et al.*, 2004b). Coronary wedge pressure is the distal pressure when anterograde flow is totally occluded and represents combined collateral and venous pressure (Figure 2.8).

Figure 2.8 Schematic of coronary circulation.



Aorta is represented on the left. Pa, aortic pressure; Pd, distal coronary pressure; Pw, coronary wedge pressure; Pv, venous pressure; Qcor, coronary flow; Qcoll, collateral flow; Qmyo, myocardial flow, as shown in Figure 2.8.

Figure 2.9 Calculation of $IMR_{corrected}$

Myocardial resistance = pressure/Q_{myo}

$$Qmyo = Qcoll + Qcor$$

$$FFRmyo = \frac{Qmyo}{QmyoN} = \frac{pd - pv}{pa - pv}$$

$$FFR\,cor = \frac{Qcor}{QcorN} = \frac{pd - pw}{pa - pw}$$

$$Qcor \approx \frac{1}{Tmn}$$

$$IMR = \frac{pd - pv}{Qcor} = (pd - pv).Tmn$$

This is true if Qcor = Q myo
As stenosis increases Qcoll increases Qcor decreases and Pd decreases

$$IMR\ true\ =\ \frac{Pd-Pv}{Qmyo}$$

By multiplying both denominator and numerator by QcorN and Qcor, the following equation can be derived

$$= \left[\frac{Pd-Pv}{Qcor}\right] x \left(\frac{Qcor}{Qcor\ N}\right) x \left(\frac{QcorN}{Qmyo}\right)$$

$$= \left[\frac{Pd-Pv}{Qcor}\right] x\ (FFRcor)\ x \left(\frac{QmyoN}{Qmyo}\right)$$

$$since\ QmyoN = Qcor\ in\ normal\ vessels$$

$$= \left[\frac{Pd-Pv}{Qcor}\right] x \left[\frac{FFRcor}{FFRmyo}\right]$$

$$= [(Pd-Pv)\ x\ Tmn] \left[\frac{FFRcor}{FFRmyo}\right]$$

$$= [(Pd-Pv)\ x\ Tmn] x \frac{\left[\frac{Pd-Pw}{Pa-Pw}\right]}{\left[\frac{Pd-Pv}{Pa-Pv}\right]}$$

$$= (Pd-Pv)\ x\ Tmn\ x \frac{Pd-Pw}{Pa-Pw} x \frac{Pa-Pv}{Pd-Pv}$$

$$= Tmn\ x \frac{Pd-Pw}{Pa-Pw} x\ (Pa-Pv)$$

$$assuming\ Pv\ is\ close\ to\ zero$$

$$= Tmn\ x\ Pa\ x \frac{Pd-Pw}{Pa-Pw}$$

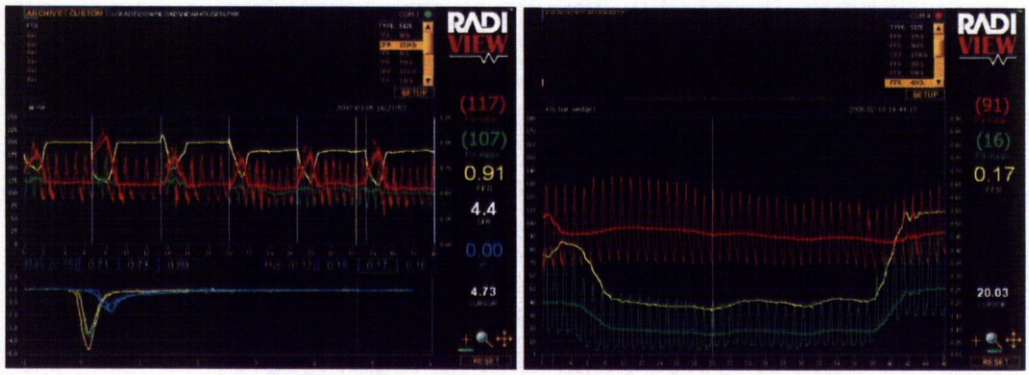
Pa,aotic pressure; Pd, distal coronary pressure; Pw,coronary wedge pressure; Pv,venous pressure; Tmn,mean transit time; Qcor,coronary flow; Qcoll,collateral flow; Qmyo,myocardial flow(Aarnoudse et al., 2004a).

Thus we have decided to use IMR over CFR due to independence from haemodynamic factors and resting conditions. Simple IMR defined as P_d x T_{mn} is an adequate method

in patients with no epicardial stenosis since coronary flow can be assumed to be equal to myocardial flow. However in our sample group, all patients have significant single vessel disease and thus will have some degrees of collateralisation and MR will be overestimated if collateral flow is not taken into account.

For the reference vessel we opted to use $IMR_{uncorrected}$ since the contribution from collateral flow in normal vessels is assumed to be zero, hence P_w is negligible. For measuring MR in the target vessel we have used $IMR_{corrected}$ since due to the presence of haemodynamically significant lesions there will some degrees of collateralisation, we would expect greater degrees of collateralisation in the more severe stenosis. It may be argued $IMR_{uncorrected}$ can be used in the target vessel once the lesion has been treated. To test this assumption we opted to repeat the measurement of wedge pressure 5 minutes post original P_w measurements in a sample of patients.

Figure 2.10 Assessment of IMR



Data from thermodilution curves at rest (yellow) and hyperaemic (blue), arterial pressure red, distal pressure (green) (left). Assessment of wedge pressure (green) (right). Red pressure tracings=P_a; green pressure tracing=P_d.

2.2.2 Assessment of MR in this study

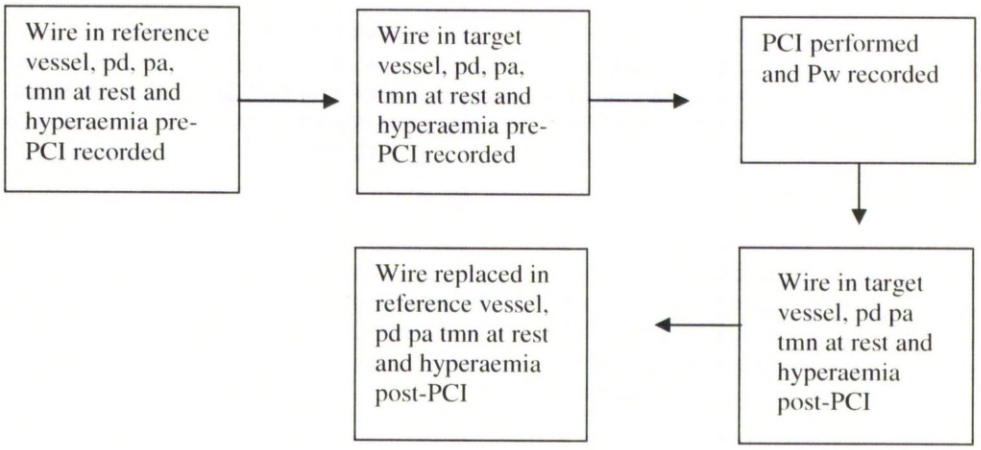
All subjects were fasted from 6 am on the morning of the procedure and allowed clear fluids until the angioplasty was performed. All patients were given with 600mg aspirin and 600mg clopidogrel orally, 12 hours before the procedure, as is standard practice for patients undergoing PCI to reduce the incidence of acute thrombosis within the stent, and all normal medications were continued. Prior to PCI a 16G cannula was inserted into a large vein in the antecubital fossa. Upon arrival to the catheter laboratory in the LHCH, the patient was connected to continuous ECG monitoring. The skin was anaesthetized close to the distal radial artery 1cm proximal to the wrist crease with 2 mls of 2% lignocaine. A radial artery introducer needle (Cook 21G 40 mm) was used access the lumen of the radial artery, once good flow of blood was visible from the lumen of the needle, a 0.018 inch introducer wire was passed into the

vessel and the needle removed over the wire. A 6F 23cm hydrophilic radial artery sheath (Flexor® Check-Flo® Introducer Sets, Cook Medical, Ireland) was inserted over the introducer wire and left in situ, the wire was removed. A standard J-tipped catheter guidewire was inserted via the radial artery sheath into the ascending aorta. 6F coronary guide catheter was advanced over the wire into the ascending aorta. An intra arterial bolus of heparin sulphate was administered at dose of 75iu/kg via the guide catheter before the left main coronary artery was engaged with the guide catheter.

The guide catheter was used to engage left main coronary artery and coronary angiogram was performed. In a separate 20ml syringe, 2mls 0.1% Isosorbide dinitrate was added to 18mls normal saline, making a 100mcg/ml solution. 200mcg of intracoronary glyceryl dinitrate was administered into the coronary artery to ensure maximal epicardial vasodilation and repeated every 30 minutes to prevent coronary spasm which can affect the haemodynamic data.

The pressure wire was calibrated, and then introduced into the guide catheter. Once the sensor reached the tip of the guide catheter in the left main coronary artery, the pressure reading from the pressure wire was equalised with arterial pressure measured from the tip of the guide catheter seated in the left main coronary artery. Care was taken to ensure there was no wedging of the guide catheter in the left main coronary artery which could give false low measurements of arterial pressure.

Figure 2.11 Haemodynamic data protocol.



The wire was first passed into the reference vessel, which was the vessel without significant disease in which no procedure was planned. Once the wire was in the distal third of the vessel then an angiogram was acquired as a reference marker for the position of the tip of the wire. This was important to ensure that for subsequent readings there had been no movement of the wire in the vessel. The guide catheter was flushed with normal saline solution for 30 seconds and baseline recordings of pressure were made in real time using the RADI analyzer. Off line analysis of data was possible once data had been recorded. At least three rapid injections of 3mls saline at room temperature were injected into the coronary artery and thermodilution curves recorded using the RADI analyser. During injections it was important to check that the guide catheter was well seated in the coronary artery ostium but not wedged. Hyperaemia was induced by infusion of intravenous adenosine at a rate of 140mcg/kg/min (De *et al.*, 2003) via a Baxter Flo-Guard 6201infusion pump, the patients arm was kept straight during infusion. The patient was advised prior to starting the infusion to

anticipate the onset of chest tightness and breathlessness, both of which are common side effects of intravenous adenosine infusion. Patients were advised to breathe normally and avoid breath holding. Following continuous two minute infusion of adenosine to achieve a state of steady state of hyperaemia three injections of 3mls saline at room temperature were repeated with the safety reservoir syringe and hyperaemic aortic and distal coronary pressures were recorded. The adenosine infusion was kept running until all these measurements were completed. Throughout these measurements great care was taken to ensure little or no movement of the tip of the wire by repeated x-ray screening to check position of the tip of the wire. At this stage resting and hyperaemic P_a, P_d and T_{mn} had been recorded in the reference vessel pre-PCI.

The pressure wire was removed from the reference vessel and equalisation with aortic pressure was checked once again before the wire was passed into the target vessel (Figure 2.11). Once the wire was passed beyond the lesion and the tip of the wire was well into the distal third of the vessel a further angiogram was repeated to be used as a reference for the target vessel.

The guide catheter was flushed with normal saline solution and baseline and hyperaemic measurements of pressure and mean transit time were recorded as described previously. This provided a set of pre-intervention haemodynamic parameters in the target vessel. The angioplasty procedure was then performed in the

standard manner. Details of the procedure including number of inflations, duration of inflations, type of stents used and complications were all recorded. During the PCI wedge pressure was measured following the first 30 second balloon inflation at hyperaemia. We repeated measurement of wedge pressure in 17 patients five minutes after first balloon inflation. Once PCI was completed and satisfactory result was obtained the position of the distal tip of the wire was checked to see if no movement had occurred from the position used to measure the pre-intervention parameters. The guide catheter was flushed with normal saline for 30 seconds. Baseline and hyperaemic measurements of aortic and coronary pressure, and mean transit time were recorded in the target vessel post-PCI as described previously. The wire was then repositioned to the reference artery to the reference point recorded earlier, once again the equalisation of the pressure wire was checked to ensure no signal drift had occurred. The catheter was flushed with normal saline solution for 30 seconds. Baseline and hyperaemic measurements of pressure and mean transit time were recorded as described earlier (Figure 2.10). From these recorded parameters, all the data for the calculation of IMR_{res}, IMR_{hyp}, CFR and FFR was available and these indices were calculated as described above.

2.2.3 Assessment of collateral flow

We calculated pressure derived collateral flow index (CFI), which is measure of collateral function (Billinger *et al.*, 2001). This was calculated by measuring the coronary wedge pressure during balloon occlusion and calculating the component of

distal coronary pressure which was derived from collaterals. The following formula was used to calculate CFI in our study.

CFI=(P_w-P_v)/(P_a-P_v)

P_w, wedge pressure; P_v, venous pressure ; P_a, aortic pressure.

We did not measure right atrial pressure in our study and estimated P_v to be 5mmHg for the purpose of calculating CFI.

Each time the wire was repositioned the calibration/equalisation of the wire was checked to ensure there had been no drift in signal. In the event of a drift in signal, a correction was made during calculations to account for the degree of drift. A final angiogram taken with the wire removed to ensure a good angiographic result. At the end of the procedure the radial artery sheath was removed and a TR band applied over the radial artery puncture site to maintain haemostasis.

All haemodynamic data were recorded digitally for off-line analysis on RADIVIEW™ software v.2.0.

2.3 Measurement of inflammatory markers and troponin-T

Blood sample was taken in all subjects before, 1 hour post and 24 hour post-PCI. 30ml of blood was taken from a vein in the arm. The blood was transferred into the corresponding collecting tubes. The samples were immediately taken to the lab and

centrifuged at 3000rpm for 10 minutes. The serum was transferred to labelled microtubes and stored at -80°C. To avoid intra-assay variation the assays were analysed in batch. All the samples were analysed in duplicate at the research laboratory LHCH expect for troponin-T, which was analysed at RLUH clinical chemistry department within 6 hours.

2.3.1 HsCRP

HsCRP assays were measured using the R and D HsCRP quantakine® ELISA kits. (R&D Systems Europe, Ltd. 19 Barton Lane, Abingdon Science Park, Abingdon, OX14 3NB) This assay utilises the sandwich enzyme immunoassay technique. A monoclonal antibody specific for CRP was pre-coated onto a microplate. The samples and standard were pipetted into the wells. HsCRP present in the samples were bound by the antibody in the plate.

Samples and standard were diluted by 100-fold by adding 10µl of sample or standard to 990µl of calibrator diluent (buffered protein base with preservative). 100µl of assay diluent was added to each well and 50µl of diluted sample or standard is added per well. The wells were covered and incubated at room temperature for 2 hours. Each well was washed with 400µl of wash buffer solution (buffered surfactant) with a manifold dispenser 4 times. Any remaining wash buffer was aspirated and the plate inverted and blotted against paper towels. 200µl of conjugate (mouse monoclonal antibody against CRP conjugated to horseradish peroxidase) was added to each well.

The wells were covered and incubated at room temperature for 2 hours. Each well was washed and aspirated 4 times and any remaining buffer blotted against paper towels. 12.5mls stabilised of hydrogen peroxide and 12.5mls of stabilised tetramethylbenzidine were mixed together 15 minutes before use to form the substrate solution. 200µl of substrate solution was added to each well. And incubated at room temperature and wrapped in foil to protect from light. After 30 minute incubation 50µl of 2N sulphuric acid was added to each well to stop the reaction.

The optical density of each well was measured using a Wallac Victor2 1420 Multilabel counter (PerkinElmer Life. Sciences, Boston, MA, USA). Results were analysed with and interpreted with Wallac1420 manager V2.0. The wavelength on the well reader was set at 450nm and wavelength correction set to 540nm.

A standard curve was created with Origin 6.0, by plotting absorbance for each standard on y-axis and CRP concentration on the x-axis, to get the line of best fit a log transformation was performed for concentration of CRP. A linear regression analysis was performed on the line of best fit and this was used to calculate the amount of detectable CRP in the samples. Since the samples were diluted 100 fold, a correction calculation was performed to give the final value of CRP per sample. All samples were performed in duplicate and the mean was used. The mean detectable minimum dose of CRP is 0.01ng/ml with this assay. The coefficient of variation was 4.4% at 4.79ng/ml and 8.3% at 18.9ng/ml.

2.3.2 IL-6

IL-6 assays were measured using the R and D human IL-6 quantakine® ELISA kit. (R&D Systems Europe, Ltd. 19 Barton Lane, Abingdon Science Park, Abingdon, OX14 3NB) This assay utilises the sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-6 was pre-coated onto a microplate. The samples and standard are pippetted into the wells. IL-6 present in the samples war bound by the antibody in the plate.

The methods used for measuring IL-6 is similar to CRP described above. The only difference was that serum samples were not diluted. The coefficient of variation of the assay was 4.2% at 16.8pg/ml and 2.0% at 186pg/ml.

2.3.3 sICAM-1

sICAM-1 assays were measured using the R and D sICAM-1 Quantakine® ELISA kit (R&D Systems Europe, Ltd. 19 Barton Lane, Abingdon Science Park, Abingdon, OX14 3NB). This assay utilises the sandwich enzyme immunoassay technique. Rat monoclonal antibody specific for sICAM-1 was pre-coated onto a microplate. The samples and standard are pipetted into the wells. sICAM-1 present in the samples were bound by the antibody in the plate.

Samples and standard were underwent dilution by 20-fold, 20µl of sample or standard added to 380µl of calibrator diluent (buffered protein base with preservative). 100µl of sICAM-1conjugate (monoclonal antibody against human sICAM-1with horseradish peroxidase) was added to each well followed by the addition of100µl of diluted sample or standard per well. The wells were covered and incubated at room temperature for 1.5 hours on a horizontal orbital microplate shaker (0.12" orbit) set at 500 rpm. Each well was washed with 400µl of wash buffer (buffered surfactant) with a manifold dispenser 4 times. Any remaining wash buffer was aspirated and the plate inverted and blotted against paper towels. 12.5mls stabilised of hydrogen peroxide and 12.5mls of stabilised tetramethylbenzidine were mixed together 15 minutes before use to form the substrate solution. 100µl of substrate solution was added to each well and wrapped in foil to protect from light. After 30 minute incubation period, 50µl of 2N sulphuric acid was added to each well to stop the reaction.

The optical density of each well was measured using a Wallac Victor2 1420 Multilabel counter (PerkinElmer Life. Sciences, Boston, MA, USA). Results were analysed with and interpreted with Wallac1420 manager V2.0. The wavelength on the well reader was set at 450nm and wavelength correction set to 540nm.

A standard curve was created with Origin 6.0, by plotting absorbance for each standard on y-axis and sICAM-1 concentration on the x-axis, to get the line of best fit a log transformation was performed for concentration of sICAM-1. A linear regression

analysis was performed on the line of best fit and this was used to calculate the amount of detectable sICAM-1in the samples. Since the samples were diluted 20 fold, a correction calculation was performed to give the final value of sICAM-1 per sample. All samples were performed in duplicate and the mean was used. Coefficient of variation is 3.6% at 4.61ng/ml and 5.0% at 19.6ng/ml.

sVCAM-1

sVCAM-1 assays were measured using the R and D sVCAM-1 quantakine® ELISA (R&D Systems Europe, Ltd. 19 Barton Lane, Abingdon Science Park,. Abingdon, OX14 3NB) This assay utilises the sandwich enzyme immunoassay technique. A rat monoclonal antibody specific for sVCAM-1 has been pre-coated onto a microplate. The technique used for measuring soluble levels of VCAM-1 is identical to sICAM-1 as described earlier. The mean CV intra assay precision is 2.3% at 583ng/ml and 3.6% at 2421ng/ml.

2.3.5 Endothelin-1

ET-1 assays were measured using the R and D ET-1 Quantiglo® ELISA kit (R&D Systems Europe, Ltd. 19 Barton Lane, Abingdon Science Park,. Abingdon, OX14 3NB). This assay utilises the sandwich enzyme immunoassay technique. A monoclonal antibody specific for ET-1 was pre-coated onto a microplate. The samples and standard are pipetted into the wells. ET-1 present in the samples was bound by the antibody in the plate.

100µl of assay diluent was added to each well followed by 100 µl of sample or standard per well. The wells were covered and incubated at room temperature for 1.5 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 rpm. Each well was washed with buffer 400µl (buffered surfactant) with a manifold dispenser 4 times. Any remaining wash buffer was aspirated and the plate inverted and blotted against paper towels. 200µl of conjugate (mouse monoclonal antibody against ET-1conjugated to horseradish peroxidase) was added to each well. The wells were covered and incubated at room temperature for 3 hours on the microplate shaker. Each well was washed with 400µl and aspirated 4 times and any remaining buffer blotted against paper towels. 8 mls stabilised of hydrogen peroxide and 4 mls of stabilised enhanced luminol were mixed together 15 minutes before use to form the working glo reagant. 100µl of working glo reagent was added to each well and incubated at room temperature and protected from light for 10 minutes. The relative light units (RLU) of each well was measured using a Wallac Victor2 1420 Multilabel counter (PerkinElmer Life. Sciences, Boston, MA, USA). Results were analysed with and interpreted with Wallac1420 manager V2.0.

A standard curve was created with Origin 6.0, by plotting absorbance for each standard on y-axis and ET-1 concentration on the x-axis, to get the line of best fit a log transformation was performed for concentration of ET-1. A linear regression analysis was performed on the line of best fit and this was used to calculate the amount of

detectable ET-1in the samples. All samples were performed in duplicate and the mean was used. The coefficient of variation was 3.3% at 1.78pg/ml and 2.6% at 90.9pg/ml.

2.3.6 sP-selectin

sP-selectin assays were measured using the R and D sP-selectin quantakine® ELISA kit (R&D Systems Europe, Ltd. 19 Barton Lane, Abingdon Science Park,. Abingdon, OX14 3NB) This assay utilises the sandwich enzyme immunoassay technique. A mouse monoclonal antibody specific for sP-selectin has been pre-coated onto a microplate. The samples and standard are pippetted into the wells. sP-selectin present in the samples are bound by the antibody in the plate. Samples and standard were diluted 20-fold by adding 15µl of sample or standard to 285µl of calibrator diluent (buffered protein base with preservative). 100µl of sP-selectin conjugate (monoclonal antibody against human soluble sP-selectin with horseradish peroxidase) was added to each well followed by addition of 100µl of diluted sample or standard per well. The wells were covered and incubated at room temperature for 1 hour. Each well was washed with 400µl of wash buffer (buffered surfactant) with a manifold dispenser 4 times. Any remaining wash buffer was aspirated and the plate inverted and blotted against paper towels. 12.5mls stabilised of hydrogen peroxide and 12.5mls of stabilised tetramethylbenzidine were mixed together 15 minutes before use to form the substrate solution. 100µl of substrate solution was added to each well and incubated at room temperature and wrapped in foil to protect from light for 15 minutes. 50µl of 2N sulphuric acid was added to each well to stop the reaction.

The optical density of each well was measured using a Wallac Victor2 1420 Multilabel counter (PerkinElmer Life. Sciences, Boston, MA, USA). Results were analysed with and interpreted with Wallac1420 manager V2.0. The wavelength on the well reader was set at 450nm and wavelength correction set to 540nm.

A standard curve was created with Origin 6.0, by plotting absorbance for each standard on y-axis and sP-selectin concentration on the x-axis, to get the line of best fit a log transformation was performed for concentration of sP-selectin. A linear regression analysis was performed on the line of best fit and this was used to calculate the amount of detectable sP-selectin in the samples. Since the samples were diluted 20-fold, a correction calculation was performed to give the final value of sP-selectin per sample. The mean CV intra assay precision is 7.9% at 1.2ng/ml and 8.8% at 12.9 ng/ml.

2.3.7 Assessment of neutrophil MAC-1 receptor CD11b/CD18 activation

Blood samples were collected into tubes containing liquid potassium EDTA (Becton Dickinson; 0.25% final concentration), mixed gently and kept at room temperature before analysis within 30 minutes. In separate tubes, 50µl of whole blood was incubated with 5µl of fluorescein isothyocyanate (FITC) labelled CD45 and 5µl of phycoerythrin (PE) labelled mouse anti-CD11b or PE labelled mouse immunoglobulin isotype control (BD Pharmingen). Cell preparations were incubated for 1 hour with

gentle mixing at 4°C followed by erythrocyte lysis and cell fixation by incubating for 10 minutes at room temperatures with equal volumes of optilyse B (Becton Coulter Ltd) followed by the addition of 500µl of distilled water and further incubation for 10 minutes at room temperature.

All flow cytometric measurements were performed using Becton Dickinson FACScalibur, flow cytometer. Day to day alignment and calibration of this instrument was achieved with commercial preparations of fluorescent micro-spheres (Coulter electronics Ltd, Luton, Bedfordshire, UK). Leukocyte populations were isolated by CD45 fluorescence and forward and side scatter dot plots. The mean fluorescence intensity (MFI) of 10,000 cells was measured and compared to that of control cells incubated with matched PE-labelled isotope controls.

Neutrophils could be distinguished from monocytes and lymphocytes by the combination of low angle forward and right angled side scattered light, a cytogram of each cell population was generated (Figure 2.12).

Figure 2.12 Graphical representation of leukocyte populations with flow cytometry

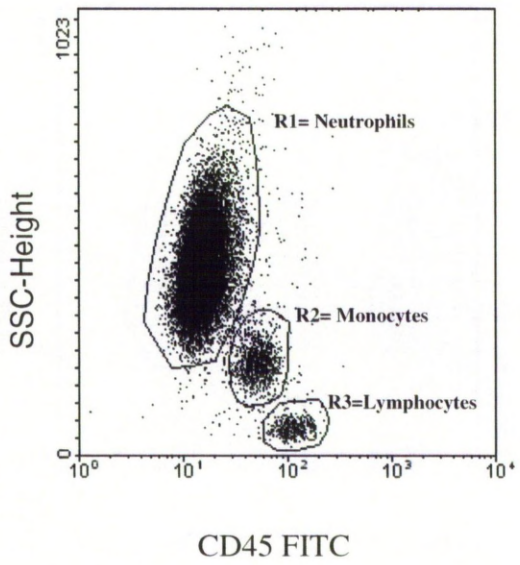
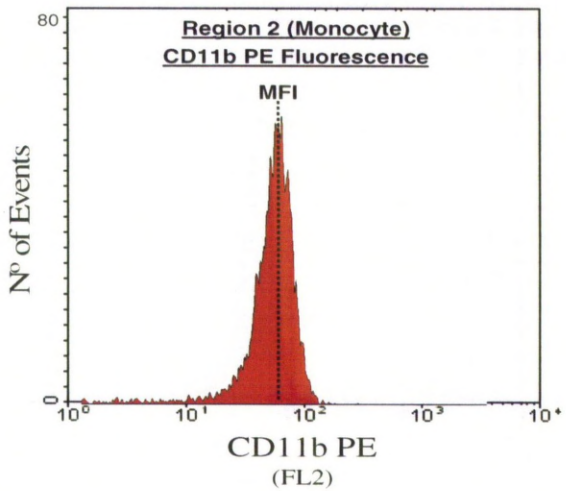


Figure 2.12 demonstrates dot plot to show separation of WBC populations based on side scatter (SSC) and CD45 FITC. Regions were drawn around the Neutrophil (R1), Monocyte (R2) and Lymphocyte (R3) subpopulations and CD11b PE fluorescence from 10,000 events collected from each region was analysed (Figure 2.13).

Figure 2.13 Mean fluorescence intensity of CD11b PE of leukocyte sub-population



Histogram to show peak CD11b PE fluorescence from a leukocyte subpopulation (eg Region 2-Monocytes) and analysis of Mean Fluorescence Intensity (MFI) as shown in Figure 2.13.

2.3.8 Measurement of cardiac troponin-T

All patients had levels of troponin T measured pre-PCI and 24 hours post PCI. In this study troponin-T was determined using the third generation electrochemiluminescent immunoassay (ECLIA) on the Roche E170 immunoassay analyzer (Roche Diagnostics, UK). The assay utilizes the 'sandwich' principle, with a biotinylated monoclonal troponin-T specific 'capture' antibody and a 'detection' antibody labelled with a ruthenium complex, which is also troponin-T specific. The upper reference limit (99th percentile) for the cardiac troponin-T assay is 0.01μg/l. However, due to poor precision of the

assay at this level, a troponin-T level with a 10% Coefficient variation or less has been used to indicate myocardial necrosis. For the assay we used, this level is 0.045μg/l. Thus for the purpose of our study we have classified peri-procedural myocardial injury as elevation of troponin-T above 0.04μg/l.

2.4 Imaging of myocardial inflammation

2.4.1 Introduction

There are many potential ways of imaging inflammation although imaging inflammatory changes in the myocardium can be challenging. Contrast enhanced MRI has been shown to detect inflammatory myocardial changes in patients diagnosed with myocarditis (Friedrich *et al.*, 1998a).

More recently it is possible to detect and quantify myocardial microvascular inflammation using microbubbles in an experimental model which can adhere to leukocytes (Lindner *et al.*, 2000b).To investigate the possible mechanisms of downstream arterial effects, we employed radio-labelling of leukocytes with subsequent visualisation by SPECT scanning. The principle of blood cell labelling is that lipophilic radiometal-chelate complexes such as Tc99 are able to enter the cell membrane of the leukocytes, once inside the Tc99 is transformed into a hydrophilic complex and irreversibly bound (Peters, 1994b). This technique requires that leukocytes are separated from intravenous blood and labelled in vitro. We decided to pursue this method due to the availability of local expertise.

2.4.2 Methods

We performed a pilot study on the first nine patients investigated. Each patient had a radiolabelled leukocyte scan performed on two occasions; the 1st 7 days prior to PCI, the 2nd 24 hours post angioplasty, to determine both baseline uptake of leukocytes in the myocardium and identify any changes following angioplasty.

2.4.2.1 Radiolabelling procedure

On each occasion a 51ml sample of blood was withdrawn from a vein in the antecubetal fossa with a 16-G butterfly needle, into a syringe containing 9 mls ACD solution (consisting of 0.73g of anhydrous citric acid, 2.2g of sodium citrate 2.45g of dextrose monohydrate in 100mls water) to prevent coagulation. The mixture of blood and acid citrate dextrose was gently mixed by turning the syringe end over end a few times.15mls of the blood-ACD solution was dispensed into a sterile centrifugation tube and centrifuged at 2000g for 10 minutes at room temperature. The cell free plasma was separated from the cell rich pellet for use later.

4.5mls of 10% 2-hydroxyethyl starch 200kDa (HES sedimenting agent) was added to syringe with the remaining 45mls of blood-ACD and gently mixed, the syringe was placed upright at room temperature, thus allowing the erythrocytes to sediment, for between 30-45 minutes. The supernatant, which consisted of platelet and leukocyte rich plasma was collected in a sterile centrifuge tube by attaching a 16-G butterfly needle to the syringe and gently injecting without disturbing the erythrocytes. This cell

rich supernatant was centrifuged at 150g for 5 minutes. This resulted in a leukocyte rich sediment and platelet rich supernatant. The supernatant was removed and the pellet was re-suspended in 1ml of cell free plasma. ^{99m}Tc-HMPAO (Ceretec, Amersham Health,UK) was freshly prepared as follows; 5ml of phosphate buffered saline containing monosodium phosphate and dibasic phosphate in 4.5ml of normal sodium chloride solution, was added to a vial containing a freeze dried mixture of 0.5mg HMPAO, 7.6μg stannous chloride dehydrate and 4.5mg sodium chloride. The vial was gently swirled for 10 seconds until complete dissolution of the powder. 1ml of the freshly prepared ^{99m}Tc-HMPAO was added to the mixed leukocyte cell suspension and incubated at room temperature for 10 minutes with gentle swirling of the tube periodically to prevent sedimentation of cells. After incubation 5mls of cell free plasma was added to the tube and centrifuged for 5 minutes at 150g. The radiolabelled pellet was resuspended in 3mls cell free plasma ready for injection.

2.4.2.2 Injection of radioisotope

The labelled cells were re-injected intravenously slowly into the patients within 1 hour of labelling with a 16-G butterfly needle. In the post-angioplasty studies, blood was taken for labelling prior to the angioplasty and the labelled leukocytes reinjected 1 hour after the angioplasty.

2.4.2.3 Imaging

Eight patients completed both the pre and post angioplasty ^{99m}Tc-HMPAO-WBC imaging protocol. In all instances, both pre & post angioplasty, SPECT images were acquired 24 hours post reinjection of the radiolabelled leukocytes on the RLUH's General Electric Medical Solutions' Infinia gamma camera system. A Low Energy General Purpose (LEGP) Parallel Hole Collimator was used for all imaging, recording for 120 seconds per view. A 180° semicircular orbit was used, with 6° per view. An image matrix of 128 x 128 pixels was used with a pixel size of 3.32mm.

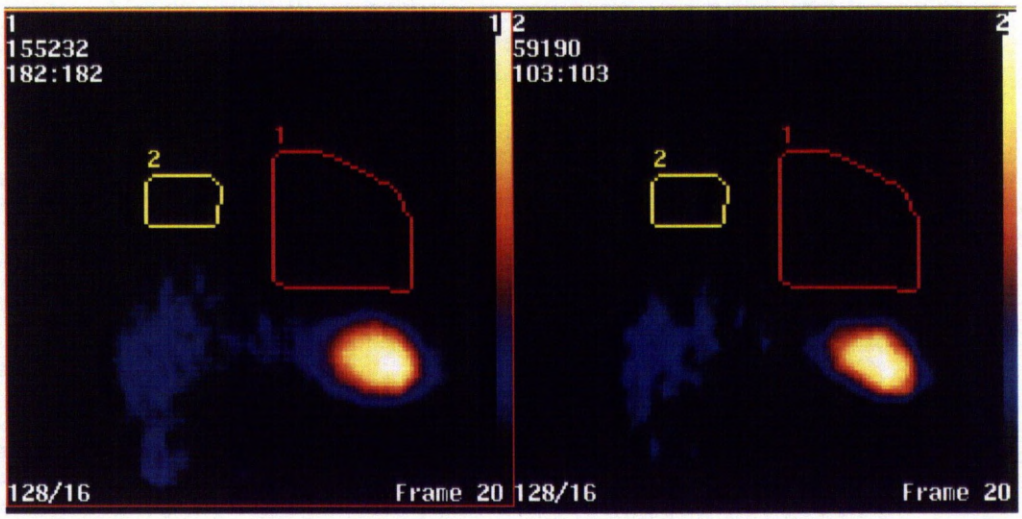
2.4.2.4 Quantification

A physicist in nuclear medicine, who was unaware of the clinical details of each patient graded the planar and SPECT images as positive (uptake of ^{99m}Tc-HMPAO-WBC clearly seen in the myocardium) or negative, and performed the quantification of uptake described below.

Leukocytes do not normally localise in the myocardium, but do localise in the spleen and (to a lesser extent) the liver. There is also a small quantity localised in bone marrow, including the sternum. The quantification is based on the analysis of background corrected uptake observed in the region where the heart would be visualised if a tracer with myocardial uptake was used. To generate slices containing this volume, the following algorithm was applied; Both the baseline and post-PCI data sets were processed with filtered back projection (FBP) SPECT reconstruction using a high resolution metz filter with order = 40 on the Nuclear Diagnostics (NUD)

HERMES processing station. The coronal data sets for both studies were created and stored. Using the NUD Multimodality software, the coronal data sets were registered and aligned together. The coronal slices posterior to the sternum and transverse slices superior to the spleen were summated (The same slices summated for both baseline and post-PCI datasets to ensure approximately the same anatomy was compared).The myocardial ROI was defined to be the region in the chest to the left of the midline, superior to the spleen/liver and posterior to the sternum. This ROI includes the myocardium and some adjacent non-myocardial anatomy. A background ROI was chosen contra-lateral to the myocardial ROI. An example of the ROIs applied is seen in Figure 2.14. The ROI from the baseline study were copy and pasted onto the post-PCI summated image to reduce the variation arising from manually defined ROIs between post and pre scans. Uptake of ^{99m}Tc-HMPAO-WBC was calculated in the myocardial and background ROI as follows; The total number of counts was divided by the number of pixels in the ROI to give counts/pixel. Each pixel was a summated frame of 27 voxels, each voxel measures 3.2mm x 3.2mm x 3.2mm. Hence we were able to calculate counts/mm^3 for both regions. We subtracted counts/pixel myocardial from counts/pixel background to show the overall difference in uptake in the myocardial region compared with the background ROI. We then compared the difference between post-PCI$_{(ROImyo-ROIbackground)}$ – pre-PCI$_{(ROImyo-ROIbackground)}$.

Figure 2.14 ROI analysis of summated data set



Example of ROI placement on summated anterior coronal slices pre-PCI (left) post-PCI (right) as seen in Figure 2.14. The bright uptake seen in both images on the right represents uptake in the spleen. Some uptake is seen in each image opposite to the spleen, this represents uptake in the liver. The red box represents myocardial ROI and the yellow box represents background ROI.

2.4.2.5 Quality control

The periodic intrinsic uniformity and centre of rotation quality control tests were also successfully performed to ensure adequate system performance during the period of this research trial.

2.5 Statistics

2.5.1 Power calculations

The power calculation was based on two measures per individual—a measure of microvascular function on both the unaffected vessel and the target vessel, and a measure of inflammatory activation. These were obtained both pre and post intervention. There were two main analyses: a regression analysis using pre-intervention (baseline) inflammatory markers as predictors of the post-intervention microvascular function, and a correlation analysis of the pre–post change in microvascular function versus pre–post change in inflammatory marker measures. To determine the requirement for patient numbers, a power analysis based on correlation was carried out. On the null hypothesis of no correlation between microvascular function and inflammation, with 80% power at the 5% significance level, to detect a correlation of 0.5 would require 30 subjects, and a correlation of 0.4 would require 48 subjects, assuming normally distributed data. We therefore elected to enrol approximately 50 patients to the main body of the study.

2.5.2 Statistical analysis

Data was checked for normality and reported as mean+/–SEM if normally distributed or median and inter quartile range if not normally distributed. Comparisons between baseline factors was performed with either χ^2 or Fisher's exact test as appropriate. Comparisons were made between pre and post-haemodynamic parameters in the same individuals with Student's paired t-test or Wilcoxon rank as appropriate. Comparisons

between unpaired data were made with unpaired t-test or Mann-Whitney U test as appropriate. Results were classed as significant if $P<0.05$. Correlation co-efficient were calculated using Pearson's co-efficient or Spearman's rank as appropriate. To obtain normally distributed data IMR was naturally log transformed. Multivariate analysis of linear regression was performed using change in IMR as the dependent variable. Those variables in the univariate analysis with $P<0.25$ were included in the multivariate linear analysis using backward selection procedure. Data analysis was performed using SPSS statistical software version 17.0 (SPSS Inc. Chicago Illinois).

Chapter 3 Screening and recruitment

3.1 Introduction

Interventional studies by their nature involve complex invasive procedures, which even in non-research clinical setting are associated with many anxieties for patients. Additional procedures required for the study add to the uncertainty. Recruitment is further complicated by the fact that patients have recently been diagnosed as having ischaemic heart disease and have been told they require an invasive procedure (coronary angioplasty) which in itself has risks of 0.5% of major complications such as myocardial infarction and stroke. Furthermore the nuclear imaging arm of the study, which involved the administration of a radioactive isotope, adds anxiety for the subjects. The purpose of this chapter is to describe the screening and recruitment process of the study and describe the background characteristics of patients.

3.2 Methods

3.2.1 Screening process and patient selection

In order to identify subjects who would be suitable for inclusion into the study we screened patients from the pooled referral list for PCI at the Liverpool Heart and Chest Hospital. If the referral letter suggested single vessel PCI and no exclusion criteria were apparent from the referral letter then the patient was selected for making contact regarding provision of a patient information leaflet.

3.2.2 Making patient contact

The selected patients were approached via telephone call to ask if they would be interested in receiving a patient information leaflet and if they would be willing to consider inclusion into the study. Following a period of at least 48 hours, the patient was contacted again and asked if they would consider meeting for further discussion regarding study. The patients who agreed were met by myself in the pre-assessment clinic and the rationale for the study was explained as well as exactly what would be involved for the patient and potential risks involved in taking part in the study. During this visit, inclusion and exclusion criteria were confirmed. If the patient agreed to partake in the study then written informed consent was obtained. Medical history, Canadian cardiac society (CCS) angina score and cardiovascular examination were performed and recorded.

Table 3.1 Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
Single vessel LAD or circumflex lesion	ACS/MI in last 3 months
Indication for PCI	STEMI anytime
Age >18	Multivessel disease
	Previous CABG
	Previous PCI
	Serum creatinine >200μmol/l

	Asthma or severe COPD
	Short left main stem or separate origin of LAD or circumflex artery
	Chronic total occlusion of the target vessel
	Left ventricular ejection fraction less than 40%
	Chronic inflammatory disease

3.2.3 Justification of inclusion /exclusion criteria

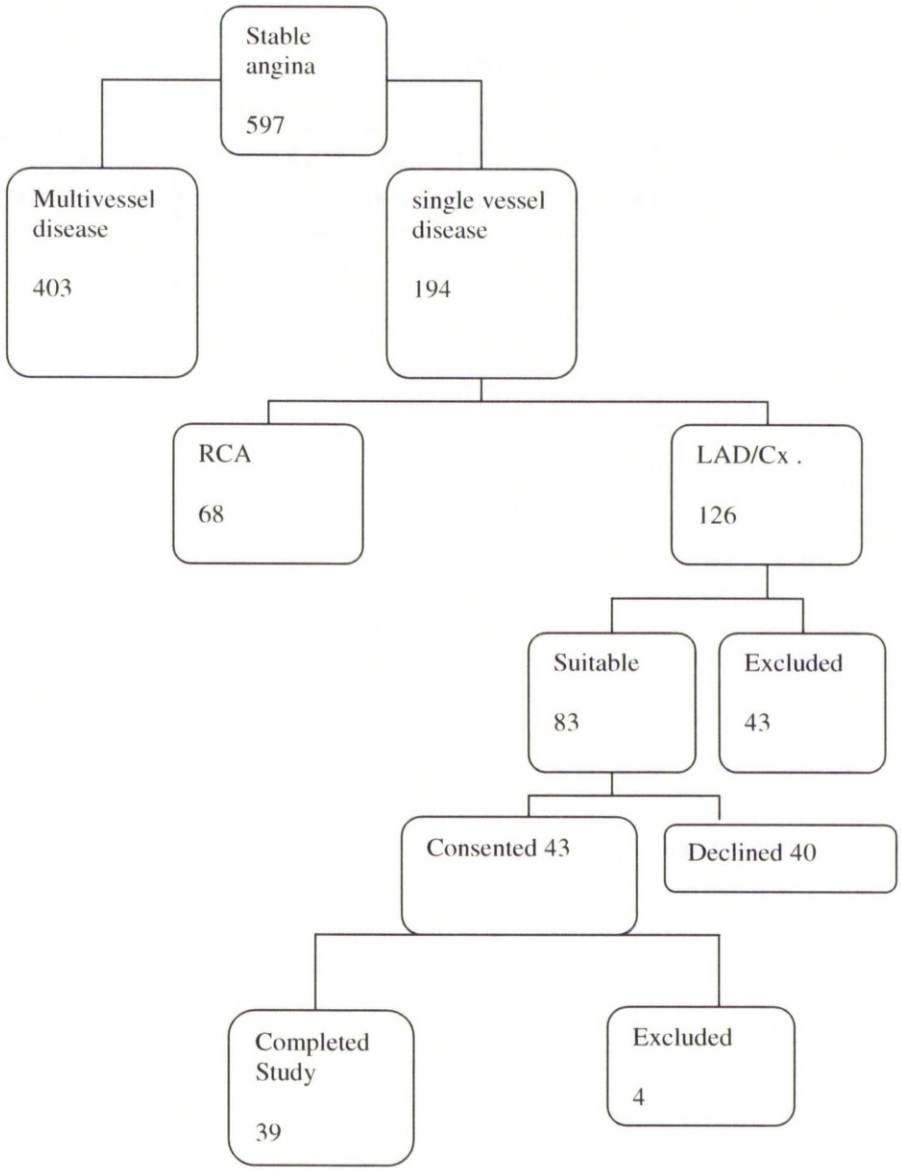
Table 3.1 summarises the inclusion/exclusion criteria for the study. Patients with AMI, or acute coronary syndrome in the last three months were excluded due to the known effects of microvascular dysfunction and the inflammatory response to infarction in this group of patients. Patients with LVEF<40%, or whom had suffered STEMI or had evidence of akinetic areas on ventriculography or echocardiography were excluded, since there would be marked microvascular abnormalities in the region of myocardium affected by the infarct. Patients with asthma and severe COPD were excluded in view of the contra-indication of adenosine use, which is required for hyperaemia. Patients with angiographic significant two vessel disease were excluded since we needed patients in whom single vessel PCI was indicated. Patients with short left main stem or separate origin of LAD and circumflex artery were excluded since good engagement of the left main stem was required for accurate measurements of mean transit time of bolus of saline injected down the left main stem. Patients with active inflammatory

disease requiring the need for immunosuppressive drugs were excluded because of potential interaction with the inflammatory response to PCI. Patients with serum creatinine >200μmol/l were excluded due to increased risk of contrast induced nephropathy. Chronic totally occluded coronary arteries were also excluded in view of the inability to obtain accurate pre-PCI mean transit times.

3.3 Results

3.3.1 Screening process

Figure 3.1 Flow diagram of the screening process



The screening process lasted from 39 months from July 2006 till September 2009. We screened 597 patients with stable angina who had undergone coronary angiography and were referred for PCI, of these 194 patients were identified with single vessel disease. Out of 194, 124 patients had either left anterior descending or circumflex artery stenosis. 43 patients were excluded due to the presence of one or more exclusion criteria. This left 83 patients eligible for the study, of which 40 declined to partake, hence 43 patients were consented (Figure 3.1).

Of the 43 patients who were included in the study 4 patients were excluded during the study, 1 patient had ventricular fibrillation immediately following passing the wire through the target lesion, one patient was found to have a haemodynamically insignificant lesion (FFR>0.8) and hence no PCI was performed, one patient had significant lesion in reference vessel territory thus requiring two vessel PCI and one patient had dissection of target vessel and was excluded due to a lengthy procedure.

Out of 39 patients present to completion, there was no complete reference vessel data in 5 patients, 2 of which were unable to tolerate the final infusion of adenosine. In another 2 patients we were unable to get a stable thermodilution curve. This was because in both cases the reference vessel was a small circumflex artery and hyperaemic mean transit time was unrecordable since this was less than the lowest level detectable by the wire. In one patient there was malfunction of the pressure wire for the final measurements. 4 patients have no complete target vessel data due to

unrecordable T_{mn} which was a result of low amplitude of the thermodilution curve in 3 patients, in all of these cases the underlying cause was very tight stenosis of the coronary artery in question. In one patient we were unable to pass the pressure wire through the stenosis and had to use a standard angioplasty wire to negotiate the lesion. All 39 patients had single de-novo lesion in either the LAD or circumflex territory, the clinical angiographic and procedural characteristics are defined in Tables 3.2-3.4. Flow cytometry was performed in 34 patients.

3.3.2 Background characteristics

Table 3.2 Cardiac risk factors

	n=39
Diabetes Mellitus	23.1%
Diabetes Mellitus (On insulin)	2.4%
Hypertension	64.1%
Hyperlipidaemia	97.4%
Smokers current	17.9%
Smokers ex (quit in last12/12)	23.1%
Family history of IHD	61.5%

The majority of patients were male (69.2%), mean age of subjects was 58.2 years and all patients were symptomatic with angina. 23.1% of patients had diabetes and of these only 1 patient was on insulin (3%), 64.1% of were hypertensive on treatment and most patients were diagnosed with pre-existing hyperlipidaemia (97.4%). 61.5% of subjects had positive family history of IHD (Table 3.2).

Table 3.3 Medication history of patients prior to PCI

Drug Therapy	n=39
Aspirin	94.8%
Clopidrogel	10.3%
Beta blockers	74.4%
Long acting nitrates	35.9%
Calcium channel antagonists	48.7%
Nicorandil	23.1%
Statins	94.9%
Angiotensin converting enzyme antagonists	46.2%
Angiotensin II receptor antagonists	10.3%
Diuretics	12.8%

Drug history is outlined in Table 3.3. The background characteristics and drug history suggest a population with multiple risk factors and on multiple drug therapy for ischaemic heart disease.

Table 3.4 Functional classification of the severity of angina of patients

Canadian cardiac society angina score	n=39
I Angina occurs with strenuous, rapid or prolonged exertion.	10.3%
II Slight limitation of ordinary activity.	48.7%
III Marked limitations of ordinary physical activity.	41%
IV Inability to carry on any physical activity without discomfort - anginal symptoms may be present at rest	0%

Two patients were in class I CCS, these were symptomatic at a high workload and were not keen on taking anti-anginal medications. The rest of the patients were in either class II or III as shown in Table 3.4.

Table 3.5 Clinical characteristics of patients. *mean+/–SEM (95%CI).

Age*	60.49 ± 1.36 (57.73-63.24)
Height, cm*	171.95 ± 1.78 (168.34-175.55)
Weight, kg*	87.6 ± 2.27 (82.99-92.18)
BMI*	30.01 ± 0.7 (28.59-31.44)
Pulse rate*	62.76 ± 1.85 (59.0-66.5)
Systolic blood pressure, mmHg*	137.4 ± 3.3 (130.6-144.1)
Diastolic blood pressure, mmHg*	71.3 ± 1.7 (67.9-74.6)
Haemoglobin, g/dl*	13.81 ± 0.19 (13.41-14.16)
White cell count , $x10^9/l$*	8.01 ± 0.21 (7.57-8.44)
Neutrophil count, $x10^9/l$	4.90 ± 0.17(4.49-5.31)
Total cholesterol, mmol/l*	4.01 ± 0.14 (3.74-4.29)
LDL cholesterol, mmol/l*	1.92 ± 0.09 (1.77-2.13)

Summary of baseline clinical characteristics are outlined in Table 3.5.

3.3.3 Evidence of reversible ischaemia pre PCI

Most patients referred for angioplasty for stable angina present to the hospital in the rapid access chest pain clinics following referral from their GP. The majority of patients undergo some form of non invasive testing to differentiate between cardiac and non cardiac chest pain. The rapid access chest pain clinic provides a prompt

consultation with a cardiologist and a nurse practioner, during this consultation there is the opportunity to perform a functional assessment of cardiac ischaemia and an assessment often in the form of an exercise tolerance test (ETT). In our data set, out of 39 patients, 34 patients had ETT, of which 31 were positive for evidence of reversible myocardial ischaemia and three were negative. 3 patients of these 34 who were positive, also had myocardial perfusion scan performed with which was also positive. 2 out of the 3 patients with negative ETT had a myocardial perfusion scan which was negative for inducible ischaemia. One of patients who had negative ETT had a stress echo which was negative. Two patients had myocardial perfusion scans who were unable to tolerate ETT and three patients had no test of reversible ischaemia pre procedure (Figure 3.2).

Figure 3.2 Flow diagram representing evidence of reversible ischaemia in the study population

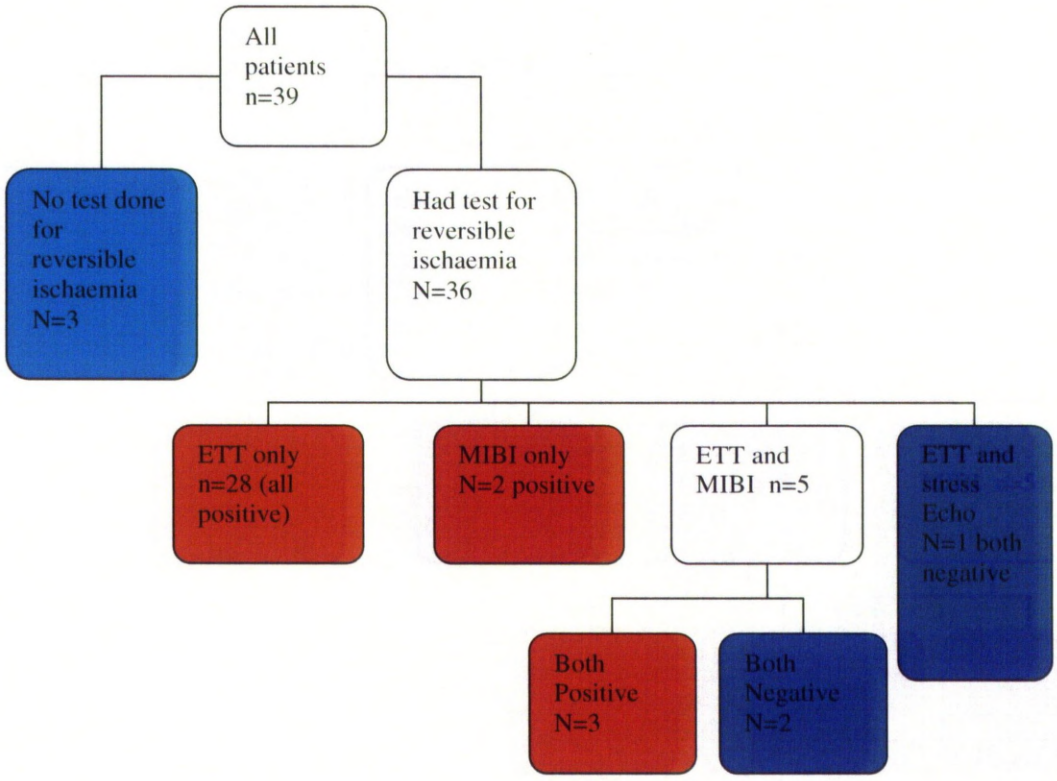


Figure 3.3 Location of coronary artery lesion

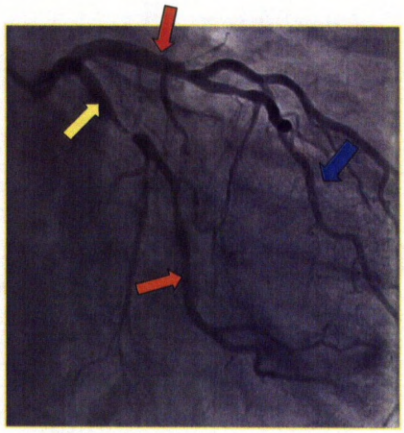


Figure 3.3 and Table 3.6 demonstrate the pattern of coronary artery disease in our study population.

Table 3.6 Distribution of location of target lesion for PCI

Proximal LAD		**43.6 %**
Mid LAD		**33.3%**
Circumflex		**20.5%**
Obtuse marginal		**2.6%**

3.4 Discussion

This chapter describes the task of studying patients for a complex interventional study with coronary artery disease and the baseline characteristics of the study population. Recruiting patients into clinical interventional cardiology studies is a challenging task, we found it particularly difficult to meet the entry criteria since most patients at

presentation had multi-vessel disease. During the recruitment period there were some important changes in the trends of management of IHD particularly the threshold at which referral for revascularisation is made. The recent Courage study compared optimal medical management vs. PCI for treatment of stable angina, (Boden *et al.*, 2007). The study suggested there was no difference to rates of death, myocardial infarction and other cardiovascular events between PCI, but there is a significant increase in freedom from angina in the PCI group. We suspect this study may have changed practice and led to a decrease in the referral rate for PCI in stable angina patients. Similar trends have been confirmed from a large PCI registry in the US (Ahmed *et al.*,) As with previous studies our data had similar prevalence of cardiac risk factors. Furthermore our study population was a well medicated group with a very high incidence of statin use. The vast majority of patients were significantly symptomatic and had evidence of ischaemia on functional testing.

Conclusion

Screening patients with IHD who have had coronary angiogram for inclusion into interventional studies is a difficult undertaking, the majority of the non-selected population has multivessel disease and a significant proportion have had recent MI, although this practice has now changed with the majority of patients with MI having coronary angiogram performed acutely followed by PCI if indicated. In addition the difficulties increase when a complex protocol was discussed and patients prefer to

undergo a relatively simpler procedure without inclusion into the study due to anxieties regarding the procedure itself.

Chapter 4 Effect of PCI on coronary and microvascular physiology

4.1 Introduction

There is growing interest in myocardial microvascular function particularly in relation to revascularisation procedures such as coronary artery bypass grafting (CABG) and percutaneous coronary angioplasty (PCI), since microvascular patency is now recognised as one of the key factors which may be important for a favourable outcome. Impairment of the microcirculation despite normal epicardial flow is associated with a poor prognosis following acute myocardial infarction (Ito *et al.*, 1992b), and microvascular injury following reperfusion is a predictor of adverse outcomes (Gibson *et al.*, 2000;Yamamuro *et al.*, 2002). Despite apparently normal epicardial flow, microvascular flow can still be impaired and this is associated with adverse events (Ito *et al.*, 2004). Assessment of the microcirculation may also provide a better understanding of the no-reflow phenomenon described earlier (Kloner *et al.*, 1974b). It is becoming apparent that microcirculatory patency has a strong association with improved prognosis in many different clinical settings and assessment of this compartment could answer some of the questions which remain unanswered in relation to successful revascularisation.

The focus of revascularisation therapies has been on restoring flow through the epicardial vessel, and the concept of PCI and CABG relies on the assumption that

restoring epicardial flow will restore perfusion and hence improve myocardial function, reduce myocardial ischaemia and improve prognosis and symptoms. However, although it is widely accepted that these forms of revascularisation do improve myocardial perfusion, the assumption that restoration of epicardial flow will necessarily restore microvascular flow has been challenged (Uren *et al.*, 1993;Kern *et al.*, 1999). One could assume that if epicardial vessel patency is restored, then microvascular flow will improve, however there is some suggestion that reperfusion/revascularisation processes themselves cause microvascular dysfunction to some degree (Selvanayagam *et al.*, 2007). In the setting of true reperfusion such as in acute myocardial infarction treated with either thrombolysis or PCI, reperfusion injury, which is the end result of activation of multiple mechanisms resulting in profound microvascular damage is very common (Braunwald and Kloner, 1985). However in stable angina patients undergoing PCI, microvascular injury is less clearly defined. We are particularly interested in the effect of PCI on microvascular function in this group of patients. If PCI does cause microvascular dysfunction in stable angina then we aim to explore underlying factors which may account for this. These aims are the focus of this chapter.

Historically interest in the microvascular compartment in relation to coronary revascularisation began in the mid 80's. The concept that revascularisation can normalise microvascular function was investigated by Bates *et al*; they studied patients, who had undergone revascularisation with either balloon angioplasty or

CABG. They found CFR remains below levels seen in normal individuals but was improved compared to patients with significant CAD pre revascularisation (Bates *et al.*, 1985). The inability to normalise CFR was assumed to be secondary to diffuse atherosclerosis. Wilson *et al.* showed in up to 50% of cases CFR, when measured immediately post-PCI remains abnormal in patients undergoing single vessel PCI. There was further improvement in CFR when assessed 5 months later in patients who did not develop restenosis. Of those whom subsequently developed restenosis, a reduction in CFR was seen following PCI (Wilson *et al.*, 1988).

Zijlstra *et al.* found immediate improvement in CFR post-PCI, but this was still below normal levels, and they found a further increase in CFR at five months, they put this down to late improvement in luminal area of vessel (Zijlstra *et al.*, 1988). Abnormal CFR may have been secondary to a less than optimal result from coronary angioplasty with balloon inflation alone and the additional effect of stenting on CFR was assessed by Kern *et al.* CFR was measured pre and post balloon angioplasty and post stenting, they found stenting improved CFR more than balloon angioplasty alone, yet despite stenting, CFR failed to normalise in a third of subjects (Kern *et al.*, 1999). However the addition of stenting the coronary artery does not always seem to further improve CFR when compared with balloon angioplasty alone (van Liebergen *et al.*, 1998). Persistently abnormal CFR following PCI was thought to be also secondary to generalised underlying microvascular dysfunction as defined by an abnormal CFR in the reference vessel pre-PCI (Kern *et al.*, 1999). An increase in basal flow was seen as

a common factor responsible for reduced CFR in many of these studies. Although a picture was emerging of some degree of microvascular dysfunction immediately following angioplasty, the medium to long term effects of PCI on MR was less well defined.

Manyari *et al.* found an immediate microvascular dysfunction (lack of normalisation) followed by a medium to long term improvement back to normal levels following PCI. They showed the persistence of perfusion defects post-PCI in patients with single vessel disease one week post-PCI, but when reassessed at three months there had been a full recovery (Manyari *et al.*, 1988). Conversely, data from patients undergoing PCI for single vessel disease, showed persistent impairment of microvascular function at 3 months with intracoronary Doppler and PET (Uren *et al.*, 1993a). Concordant short term results were found by subgroup analysis of the DEBATE study, 50% of patients had abnormal CFR immediately post-PCI and this was associated with increased revascularisation and angina at 6 months. Once again lack of normalisation of CFR was attributed to an increase in basal flow, in addition patients with abnormal CFR in the reference vessel was predictive of poor outcome post-PCI (Piek *et al.*, 2001).

The much larger DEBATE II study showed that over a third of patients had impaired CFR post-PCI despite a normalisation of the epicardial obstruction, those with abnormal CFR once again demonstrated increased baseline flow (Albertal *et al.*, 2002). In this study impaired CFR was associated with raised CKMB, suggesting that

periprocedural myocardial injury and necrosis may account for the increase in basal flow due to the release of endogenous adenosine (Hori *et al.*, 1986). Patients with CFR<2.5 immediately post-PCI had a worse prognosis as demonstrated by an increase in major adverse clinical events (MACE) at 30 days. However if the early complications were excluded then there was no difference in longer term prognosis (Albertal *et al.*, 2002). Hence the authors suggested procedural factors related to the PCI were responsible for MACE.

All the above studies demonstrated impaired CFR post-PCI in predominantly stable angina patients. There seemed to be a common finding of an increase in basal flow which was a factor in post procedural reduction in CFR. Given that CFR is a less than ideal measure of microvascular function as discussed earlier, researchers have used the concept of minimal microvascular resistance, which is defined as microvascular resistance at maximal hyperaemia. Initially a small number of studies used the Doppler catheter and wire to assess MR_v in patients undergoing PCI.

Chamuleau *et al.* assessed MR before and after PCI using hyperaemic minimal MR (MR_v) with the Doppler wire. They were able to show firstly that vessels with severe stenosis had greater MR_v in the territory supplied by the diseased artery, and by performing PCI, MR_v improves. However when the data was analysed more closely, in 25% of patients, there was either an increase in MR_v or little change compared to pre-PCI levels (Chamuleau *et al.*, 2003a). A further study demonstrated a 34% reduction

in MR_v in response to PCI, the authors suggested that PCI increased distal pressure and hence flow, thereby reducing microvascular resistance (Verhoeff *et al.*, 2005). More recently an elegant MRI study indicates that in up to 50% of cases there is evidence of distal microvascular injury post-PCI, detected by delayed enhancement with MRI. This corresponds with reduced CFR in the same area; again suggesting distal microvascular injury is the underlying cause. The authors speculate that this was due to distal embolisation, however any other cause of microvascular disturbance could have resulted in a similar pattern of microvascular damage (Selvanayagam *et al.*, 2007).

The conclusion from these studies was that a very substantial proportion (30-50%) of patients undergoing coronary intervention either have persistent microcirculatory dysfunction or in some cases develop a worsening of microcirculatory function to some degree, which in some cases is associated with poor outcome. Yet despite the clinical implications and importance of microvascular dysfunction secondary to PCI, in the stable angina patients, the effect of PCI on microvascular function is poorly defined and underlying mechanisms are still poorly understood.

This chapter describes the findings of haemodynamic physiology in relation to PCI in our study population, particularly investigating the microvascular compartment in two vessel territory to gain an insight into the factors which affect MR in stable angina.

The use of IMR to assess the effect of PCI on MR had not to our knowledge been performed prior to the start of this project. We must also take into consideration the contribution of collateral flow when measuring microvascular function. Collateral component of myocardial flow cannot be ignored or assumed to be insignificant as discussed earlier in Chapter 2, particularly when we are looking at vessels with severe stenosis (Aarnoudse *et al.*, 2004c). The greater the stenosis we would expect more collateralisation and hence a very significant proportion of myocardial flow would be collaterally derived. Therefore we set out to investigate the microcirculation from a different perspective using the novel recently described method of IMR and corrected IMR which takes into account the contribution of collateral flow (Aarnoudse *et al.*, 2004a;Fearon *et al.*, 2004a).

4.2 Methods

For a detailed description see Chapter 2 page 60. In brief, 39 patients with single vessel CAD symptomatic with angina scheduled for elective PCI were included in the study. All patients were given aspirin 600 mg and clopidrogel 600mg at least 12 hours before the PCI and normal medications were continued. PCI was performed from the right radial artery using 6-F guiding catheters. All patients received a bolus of heparin 75IU/kg at the beginning of PCI and an intra-coronary injection of 200mcg of glyceryl dintrate every 30 minutes. Each patient had measurement of P_a, P_d, T_{mn} at rest and hyperaemia in both the reference vessel and target vessel before and after PCI. FFR, CFR, IMR_{hyper}, IMR_{rest}, was calculated in both vessels as described before and

immediately post-PCI. Coronary wedge pressure was measured in all patients during 30 second balloon inflation in the target vessel. 17 patients underwent second measurement of wedge pressure 5 minutes after first recording of wedge pressure to assess acute changes in coronary wedge pressure following PCI. CFI was calculated as described in Chapter 2.

All intracoronary haemodynamic measurements were performed with RADI pressure wire® and recorded on RADI analyser® (Upsulla, Sweden). Off line analysis was performed with RADIview®. Hyperaemia was induced with IV adenosine at dose of 140μg/kg/min. PCI was performed in the standard manner. $IMR_{uncorrected}$ was calculated as $P_d \times T_{mn}$ and $IMR_{corrected}$ was calculated as follows $P_a \times ((P_d-P_w)/(P_a-P_w)) \times T_{mn}$, these measurements were all performed at maximal hyperaemia. We also calculated resting IMR in both vessels.

4.2.1 Statistical analysis

Data were checked for normality and reported as mean+/–SEM if normally distributed or median and inter quartile range if not normally distributed. Comparisons between baseline factors was performed with either χ^2 or Fisher's exact test as appropriate. Comparisons were made between pre and post haemodynamic parameters in the same individuals with Student's paired t-test or Wilcoxon rank as appropriate. Comparisons between unpaired data were made with unpaired t-test or Mann-Whitney U test as appropriate. Results were classed as significant if $P<0.05$. Correlation co-efficient

were calculated using Pearson's co-efficient or Spearman's rank as appropriate. Statistical analysis was performed with SPSS v.17 (SPSS inc. Chicago Illinois, USA). Variability between the three measurements of T_{mn} was defined as follows;

$$\mathrm{Var}(a1,a2,a3)= \max_{1,2,3} \frac{|ai-\bar{a}|}{\bar{a}}$$

4.3 Results

We recorded haemodynamic data on 39 patients. We were unable to acquire a complete set of haemodynamic data before and after the PCI in both vessels in a number of patients. Full data were not obtainable in 4 target vessels and 5 reference vessels. This was due to three cases of severe stenosis in the target vessel whereby T_{mn} was unrecordable since the lesion was so severe that not enough injectate was able to pass beyond the lesion to the sensor to get adequate signal and reading of T_{mn}. In one patient we were unable to pass the pressure wire through the stenosis and had to use a standard angioplasty guide wire to negotiate the lesion hence were unable to get readings on the target vessel and unable to measure wedge pressure. In the reference vessel we did not have full data on 5 patients, this was due to 2 patients refusal for repeat IMR in the reference vessel post-PCI due to the side effects of adenosine, inability to get good signal in two patients and a malfunction of pressure wire in one patient. Hence we had out of 39 patients, full data on target vessel in 35 patients and 34 patients in the reference vessel.

4.3.1 Procedural factors

Table 4.1 Procedural characteristics of subjects undergoing PCI.

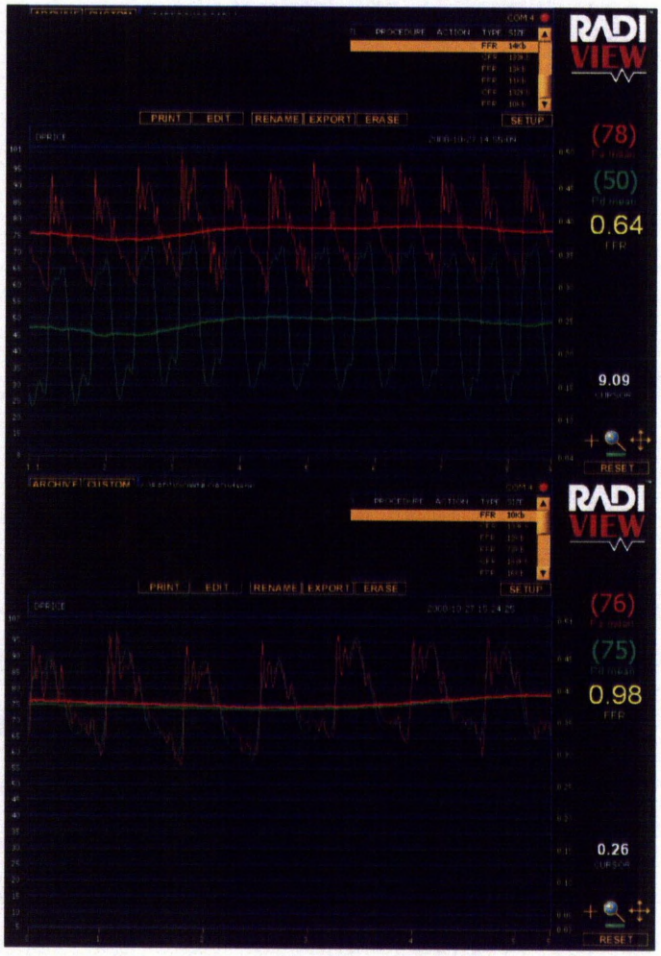
†median(IQR),*mean+/–SEM (95%CI).

No of stents †	1 (1-2)
No of inflations*	4.05 ± 0.32 (3.39-4.71)
Total time of ischaemia due to balloon occlusion during PCI*, seconds	71 ± 6.72 (54.45-84.55)
Procedure time, minutes*	98.28 ± 4.08 (89.95-106.71)
Total length of stent, mm†.	18 (15-23)
Stent Diameter, mm*.	2.88 ± 0.72

Table 4.1 describes procedural factors related to PCI in our study group.

4.3.2 General data

Figure 4.1 Haemodynamic data as measured by the pressure wire.



Tracing on top represents pre-PCI haemodynamic data in the target vessel, tracing on bottom represents post-PCI data in the target vessel as shown in Figure 4.1. Red tracing indicates aortic pressure(P_a), green tracing indicates distal coronary pressure(P_d), the number in yellow on the right hand side is the calculated FFR. P_d is

dissociated from P_a waveform and resembles ventricular pressure wave form pre-PCI. Post-PCI P_d waveform normalises and resembles aortic pressure waveform.

Table 4.2 Effect of PCI on haemodynamic parameters, comparison between the same vessel pre and post-PCI. *$P<0.05$**$P<0.01$ ***$P<0.001$

	Reference Pre (n=34)	**Reference Post (n=34)**	**Target Pre (n=35)**	**Target Post (n=35)**
P_a rest (mmHg)	97 (93-106)	100 (92-107)	100 (92.3-107)	101 (91.5-111.8)
P_d rest (mmHg)	99 (90-105)	98 (88-104.5)	**70.5 (61.8-86) *****	**100 (86-105)**
P_a hyp (mmHg)	93 (82-103)	92 (78-100.5)	94.5 (82.5-106.3)	90.5 (81.25-102.8)
P_d hyp (mmHg)	87.3 (83.3-91.4)	86 (74.5-100.8)	**.58 (45.3-65.8)*****	**83.5 (74.5-90.5)**
T_{mn} rest (seconds)	**0.73 (0.53-1.01)****	**0.57 (0.28–0.75)**	0.69 (0.52-1.1)	0.60 (0.33–0.85)
T_{mn} hyp (seconds)	**0.27 (0.19–0.35)****	**0.21 (0.17–0.27)**	**0.35 (0.25–0.74)*****	**0.20 (0.17–0.32)**
CFR	2.99+/–0.25	2.57+/–0.2	**1.96+/–0.12 ***	**2.67+/–0.24**
FFR	0.96+/–0.01	0.96+/–0.01	**0.63+/– 0.01 *****	**0.91+/–0.01**
IMR hyp uncorrected	**23.7 (15.8-31.4)****	**17.9 (12.4-24.1)**	**24.0 (14.3-35.7)***	**17.0 (13.3-24.6)**
IMR hyp corrected			14.6 (10.9-24.5)	15.9 (12.4-23.9)
IMR rest uncorrected	**72.3 (56.4-97.5) *****	**56.8 (26.7-73.1)**	47.2 (32.9-68.7)	60.0 (36.3-93.8)
IMR rest corrected			**22.6 (16.8-48.7)*****	**55.7 (29.0-87.6)**

Table 4.2 summarises all the haemodynamic data before and after angioplasty, at rest and at hyperaemia. The individual findings are discussed in detail below in the appropriate sections. (P_a rest, resting aortic pressure; P_d rest, resting distal coronary pressure; P_a hyp, hyperaemic aortic pressure; P_d hyp, hyperaemic distal pressure; T_{mn} rest, resting mean transit time; T_{mn} hyp, hyperaemic mean transit time; CFR, coronary flow reserve; FFR, fractional flow reserve; IMR $hyp_{uncorrected}$, hyperaemic index microcirculatory resistance uncorrected; IMR $hyp_{corrected}$, hyperaemic index microcirculatory resistance corrected; IMR $rest_{uncorrected}$, resting index microcirculatory resistance uncorrected; IMR $res_{corrected}$, resting index microcirculatory resistance corrected).

Variability of T_{mn}

The variability in measurements of T_{mn} was 9.34+/–0.004% for resting T_{mn} and 11.45+/–0.01% for hyperaemic T_{mn}.

Effect of PCI on heart rate

Resting heart rate increases slightly following PCI (68.7+/– 2.0 vs. 71.8+/– 1.9 P= 0.007), there is no difference in hyperaemic heart rate following PCI (86.7 +/– 2.5 vs. 87.0 +/– 2.0 P=ns).

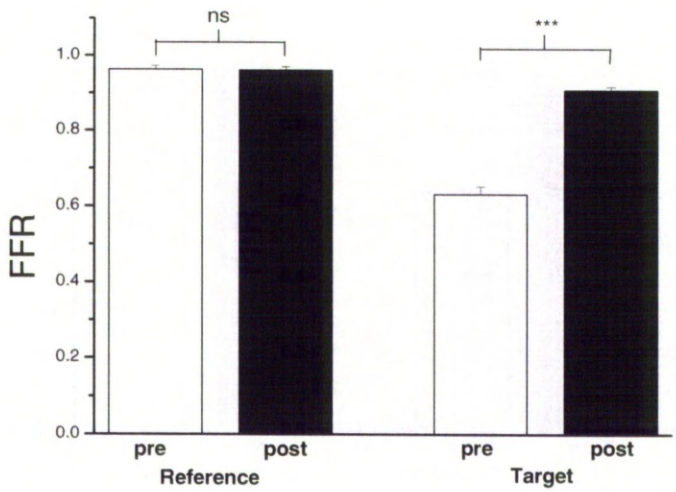
Effect of adenosine on haemodynamics

Adenosine reduces mean arterial pressure (99.5[92.3-107.5] mmHg vs. 92.5[81-104.4] mmHg P<0.001) and increases heart rate (70.1+/–2.0 vs. 86.86+/–2.2 P<0.001).

4.3.3 Fractional flow reserve and coronary flow reserve

The next set of data summaries the effect of PCI on fractional flow reserve, coronary flow reserve and index of microcirculatory resistance on both vessels following PCI.

Figure 4.2 Effect of PCI on FFR. Mean +/– SEM ***P<0.001



There was no change of FFR in the reference vessel in response to PCI, as shown in Figure 4.2 (0.96+/–0.01 vs. 0.96+/–0.01 n=34 P=0.31). We demonstrate an improvement in FFR in the target vessel post procedure (0.63+/–0.02 vs. 0.91 +/–0.01 n=35 P<0.001).

Figure 4.3 Effect of PCI on CFR measured with thermodilution. Mean +/– SEM

*P<0.05

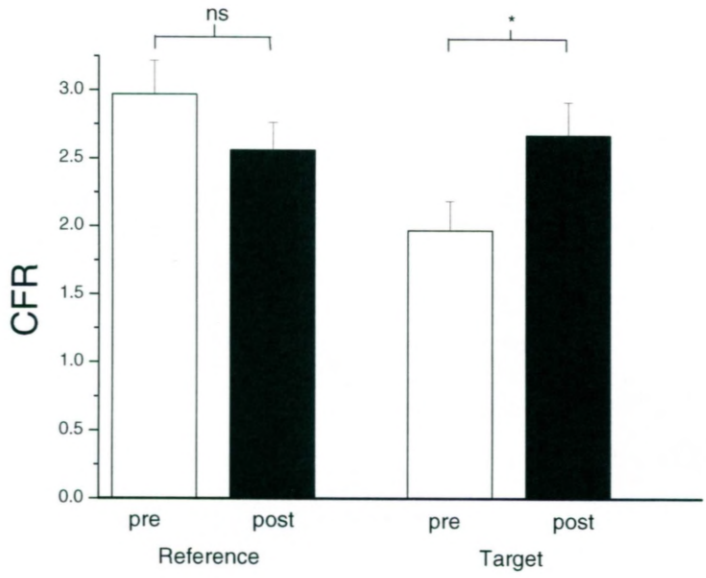
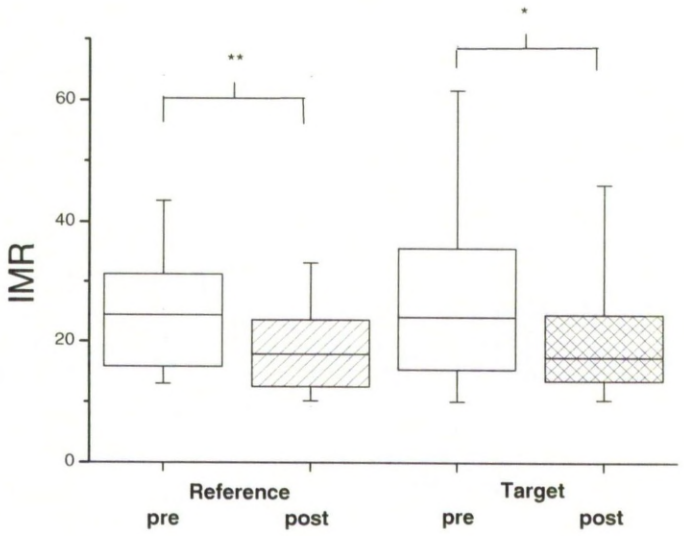


Figure 4.3 shows that there was no significant change in the CFR post-PCI in the reference vessel (2.99 +/– 0.25 vs. 2.57 +/– 0.2 n=34 P=0.08). The CFR in the target vessel increased in response to PCI (1.96 +/– 0.22 vs. 2.67+/– 0.24 n=35 P<0.05).

4.3.4 IMR

In order to investigate the effect of PCI on microvascular function we measured $IMR_{corrected}$ and $IMR_{uncorrected}$ in both vessels, before and after PCI.

Figure 4.4 Effect of PCI on $IMR_{uncorrected}$ ($IMR=P_d.T_{mn}$). Median (IQR) $*P<0.05$ $**P<0.01$



There is a significant reduction in reference vessel IMR post procedure compared with pre-PCI (23.7[15.8-31.4] vs. 17.6[12.4-24.1] n=34 $P<0.01$). There is a significant reduction in $IMR_{uncorrected}$ in the target vessel in response to PCI when collateralisation is not taken into account, as shown in Figure 4.4 (24.0[14.3-35.6] vs.17.0 [13.3-24.6] n=35 $P<0.05$).

Figure 4.5 Effect of PCI on $IMR_{corrected} = P_a.T_{mn}[(P_d-P_w)/(P_a-P_w)]$ Median(IQR). $^{**}P<0.01$

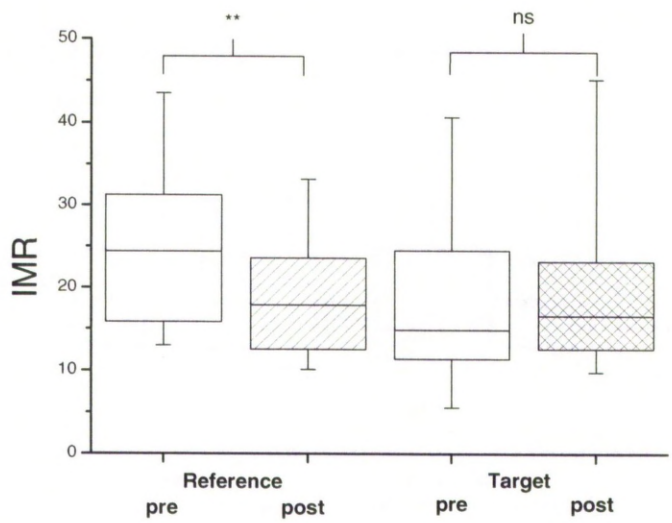
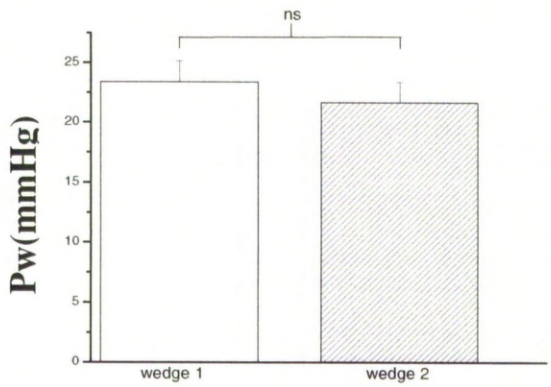


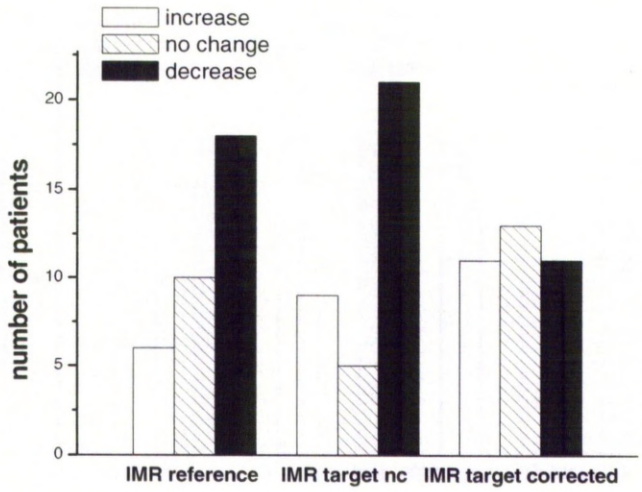
Figure 4.5 shows that when collateral flow is accounted for in the calculation of IMR, in the form of $IMR_{corrected}$, we see a heterogenous response to PCI in the target vessel. In some cases values increased, whilst in others a decrease was observed, thus overall changes were not found to be statistically significant (14.6[10.9-24.5] vs. 15.9[12.4-23.9] n=35 P=0.99). Given the heterogenous nature of the response to PCI, we analysed the degree of change in IMR from baseline to identify the group of patients who in fact had an increase in IMR following PCI. It should be noted that in the reference vessel there is no difference between $IMR_{corrected}$ and $IMR_{uncorrected}$ since we assume P_w=0 in the non diseased vessel.

Figure 4.6 Acute change in coronary wedge pressure following PCI. Mean+–SEM



There was no acute change in coronary wedge pressure when measured 5 minutes apart, as shown in Figure 4.6 (23.39+/–1.77mmHg vs. 21.67+/–1.7 mmHg n=17 *P*=ns).

Figure 4.7 Distribution of patients according to change in IMR, increase/decrease is defined as increase/decrease of >15% of baseline IMR post-PCI respectively

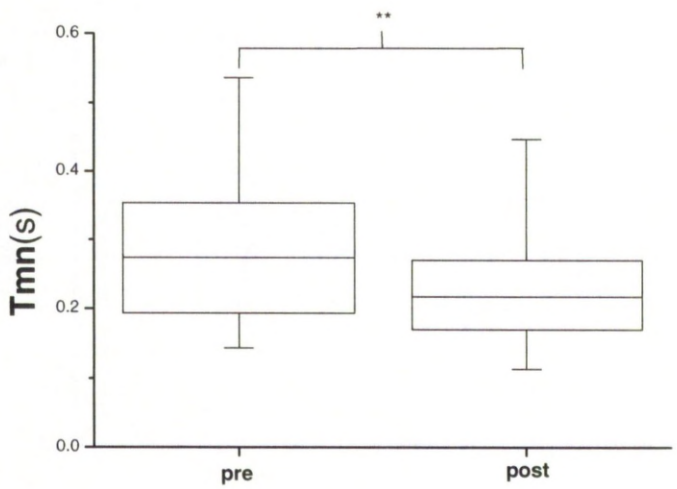


We have defined increase/decrease of IMR in response to PCI as a 15% increase/decrease from pre-PCI levels on the basis of variability of hyperaemic Tmn of 11.5% observed in our study. In the reference vessel, IMR increases in 6/34patients (17.6%), no change in 10/34 patients (29.4%) and decreases in 18/34patients (52.9%). $IMR_{uncorrected}$ increases in 9/35 patients (25.7%), decreases in 21/35 patients (60%) and is unchanged in 5/35 patients (14.3%) following PCI. 11/35 patients (31.4%) developed an increase in $IMR_{corrected}$ following PCI, there was a decrease of $IMR_{corrected}$ in 11/35 patients (31.4%) and no change in 13/35 patients (37.1%).

The IMR in the reference vessel is calculated by the same method as uncorrected IMR, since there is no epicardial stenosis and wedge pressure is assumed to be venous

pressure which is approximated to zero, hence the reference vessel IMR consists of P_d multiplied by T_{mn}. Since it could be argued that a reduction in IMR is seen due to change in baseline conditions such as blood pressure thereby accounting for the reduction in IMR in the reference vessel, therefore we assessed the individual components of P_d and T_{mn} separately.

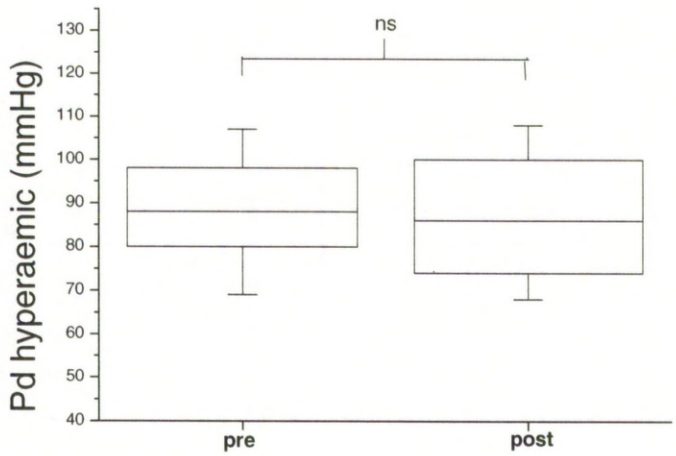
Figure 4.8 Effect of PCI on mean transit time in the reference vessel. Mean +/–SEM *P<0.05 **P<0.01



There is a reduction in hyperaemic T_{mn} post-PCI in the reference vessel territory (0.27[0.19–0.35] seconds vs. 0.21[0.17–0.27] seconds n=34 P<0.01) as shown in Figure 4.8. When we analysed changes in basal flow assessed by resting T_{mn} in the reference vessel pre vs. post-PCI, a reduction in resting T_{mn} resting is seen (0.73[0.53-1.01] seconds vs. 0.57[0.28–0.75] seconds P<0.01) There is no significant difference in resting flow in the target vessel; T_{mn} (0.69[0.52-1.1] seconds vs. 0.60[0.33–0.85]

seconds P=ns) as shown in Table 4.2. Hyperaemic flow in both vessels increases following PCI as seen by a reduction in T_{mn} in the reference vessel and target vessel respectively (0.27[0.19–0.35] seconds vs. 0.21[0.17–0.27] seconds P<0.01; 0.35[0.25–0.74] vs. 0.20[0.17–0.32] seconds P<0.001).

Figure 4.9 Effect of PCI on distal coronary pressure in the reference vessel. Median (IQR)

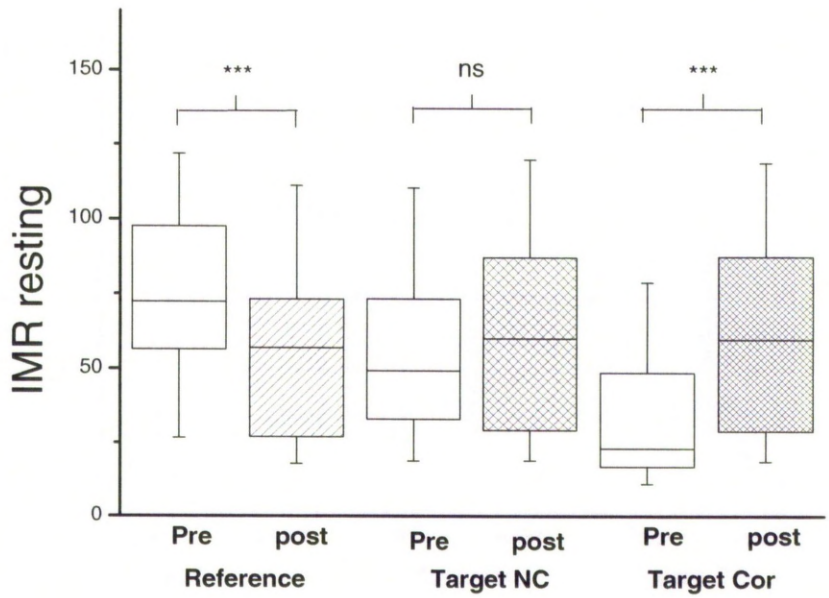


There is no change in P_d in the reference vessel following PCI (87.3mmHg [83.3-91.3] vs. 86mmHg [74.5-100.8] n=34 P=0.77), therefore the reduction in IMR is due to a reduction in the T_{mn} component of IMR.

4.3.5 Effect of PCI on resting IMR

Although $IMR_{corrected}$ is an accepted measure of microvascular function, we investigated the effect of PCI on resting IMR (without administration of adenosine) to assess how the basal microcirculation responds to PCI.

Figure 4.10 Effect of PCI on $IMR_{resting.}$ Median(IQR) ***P<0.001



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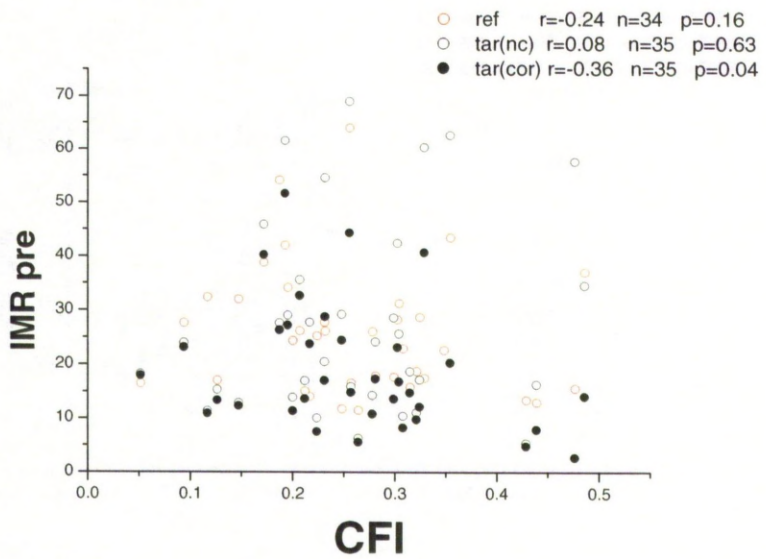
There is a decrease in resting $IMR_{uncorrected}$ in the reference vessel in response to PCI (72.3 [56.3-97.5] vs. 56.8[27.0-73.1] n=34 P<0.001). There is no significant effect of PCI on resting $IMR_{uncorrected}$ in the target vessel (48.2[33.1-73.2] vs. 56.5[29.3-87.2] n=35 P=0.18). However if $IMR_{corrected}$ is calculated for the target vessel then there is a marked increase in basal microvascular resistance (22.6 [16.8-48.7] vs. 55.7[29.0-87.5] n=35 P<0.001).

4.3.6 Collateral flow index and IMR

In order to investigate the interaction between collateral flow and microvascular function we analysed the data by comparing change in IMR vs CFI since collateral flow may be an important factor on microvascular dysfunction following PCI.

Figure 4.11. Relationship between collateral flow index and IMR



There is no relationship between CFI and IMR in either reference and target vessel when $IMR_{uncorrected}$ in used, however with $IMR_{corrected}$ there is a negative correlation as CFI increases IMR is reduced in the target vessel. R –0.36 P=0.04. Hence the greater the collateral contribution the less the IMR in the target vessel when corrected for collateral flow as shown in Figure 4.11. We also investigated the relationship between collateral flow index and FFR pre-PCI in the target vessel (R =–0.31 P=0.07).

4.3.7 Comparison of good collateral vs. poor collateral groups

Since collateral flow has been implicated by earlier studies as a potentially important factor on microvascular function and outcome of PCI, we have split the data into poor collateral (CFI≤0.2) and good collaterals (CFI>0.2) and compared clinical and haemodynamic factors.

Table 4.3 Comparison of baseline factors between patients with poor and good collateral flow. $^{**}P<0.01$

	CFI≤0.20 n=16	**CFI>0.20 n=22**
Hypertension	56.3%	72.7%
Hypercholesteraemia	87.5%	100%
Diabetes	18.7%	27.3%
Smoking history<12/12	43.8%	40.9%
Family history IHD	**81.3%**	**47.8%** **
CCS class I	12.5%	9.1%
II	62.5%	40.9%
III	25%	50%

Apart from an increased percentage of patients with a family history of IHD in the poor collateral group, there are no other differences between baseline characteristics as shown in Table 4.3.

Table 4.4 Difference in haemodynamic data pre and post-PCI in patients with poor and good collaterals

	CFI≤0.20	**CFI>0.20**
CFR ref pre PCI	2.93+/–0.47 (n =12)	3.02+/–0.32 (n=22) *P*=0.77
CFR ref post PCI	2.63+/–0.38 (n=12)	2.54+/–0.26 (n=22) *P*=0.89
CFR tar pre PCI	2.39+/–0.42 (n=16)	1.61+/–0.16 (n=19) *P*=0.07
CFR tar post PCI	2.82+/–0.40 (n=16)	2.53+/–0.30 (n=19) *P*=0.45

This table compares pre and post CFR in both vessels before and after PCI comparing poor vs. good collaterals. Overall there is no statistically significant difference between the groups, however there does seem to be a trend towards reduced CFR in the target vessel pre-PCI in good collateral group compared to the poor collateral group (2.39+/–0.42 vs. 1.61+/–0.16 *P*=0.07).

Table 4.5 Microvascular resistance before and after PCI in patients with poor and good collaterals. *$P<0.05$

	CFI≤0.20	**CFI>0.20**
IMR ref pre hyp	**27.7(24.6-37.6)* n=12**	**17.8(15.1-29.3) n=22 P=0.04**
IMR ref post hyp	**23.2(18.3-30.8) * n=12**	**16.1(11.3-21.8) n=22 P=0.02**
$IMR_{uncorrected}$ tar pre	25.8(14.6-41.7) n=16	24.9(15.5-46.2) n=19 P=0.87
$IMR_{uncorrected}$ tar post	18.0(14.7-25.8) n=16	15.73(12.5-23.7) n=19 P=0.26
$IMR_{corrected}$ tar pre	23.47(12.6-28.4) n=16	14.4(8.2-21.0) n=19 P=0.08
$IMR_{corrected}$ tar post	17.2(14.3-25.1) n=16	14.6(12.3-23.5) n=19 P=0.23

IMR in the reference vessel, both before and after PCI, is significantly less in patients with good collaterals compared to those with poor collaterals respectively (27.7[24.6-37.6] vs. 17.8[15.1-29.3] P=0.04) (23.2[18.3-30.8] vs. 16.1[11.3-21.8] P=0.02) as shown in Table 4.5. There is a trend towards lower $IMR_{corrected}$ in the good collateral group (23.47[12.6-28.4] vs.14.4 [8.2-21.01] P=0.08).

Table 4.6 Comparison of resting microvascular resistance between poor and good collateral groups. *P<0.05

	CFI≤0.20	**CFI>0.20**
$IMR_{resting}$ ref pre	86.8 (63.9-111.9) n=12	71.1 (41.0-98.4) n=22 P=0.15
$IMR_{resting}$ ref post	73.7 (28.3-108.0) n=12	54.7 (28.3-69.4) n=22 P=0.11
$IMR_{uncorrected\ resting}$ tar pre	48.3 (38.8-89.5) n=16	45.0 (22.5-70.5) n=19 P=0.26
$IMR_{uncorrected\ resting}$ tar post	60.7 (40.6-101.9) n=16	41.6 (23.1-83.2) n=19 P=0.26
$IMR_{corrected\ resting}$ tar pre	**42.2 (29.7-88.1)* n=16**	**31.3 (20.0-41.9) n=19 P=0.03**
$IMR_{corrected\ resting}$ tar post	60.3 (39.5-100.9) n=16	39.4 (21.9-82.1) n=19 P=0.23

There appears to be a trend towards a reduction in basal microvascular resistance in the good collateral group (CFI>0.2) compared to the poor collateral group (CFI≤0.20) as shown in Table 4.6. Yet this only statistically significant for $IMR_{corrected\ resting}$ in the target vessel pre-PCI (42.2[29.7-88.2] vs. 31.3[20.0-41.9] P=0.03).

4.3.7 Procedural factors and microvascular dysfunction

In order to identify factors which may be relevant in patients who develop microvascular dysfunction following PCI, we have compared baseline, procedural and haemodynamic factors with the degree of change in microvascular resistance.

Table 4.7 Correlation between procedural factors and outcome of IMR expressed as ratio of IMR post/pre-PCI for reference vessel and target vessel corrected for collateral flow. *P<0.05

	IMR ref post/pre R n=34 P		**$IMR_{corrected}$ tar post/pre** R n=35 P	
CFI	–0.01	0.96	0.14	0.43
Number of stents	0.16	0.38	0.32	0.06
Number of inflations	–0.21	–0.25	0.26	0.13
Total inflation time	–0.13	0.45	**0.34***	**0.04**
Length of stent	–0.10	0.57	0.02	0.93
Stent Diameter	**–0.39***	**0.02**	–0.16	0.36

Table 4.7 analyses the relationship between procedural factors and the degree of change in IMR in response to PCI, there is no relationship between CFI, number of inflations and length of stent used. There is a positive correlation between total inflation time of the angioplasty balloon and degree of change in $IMR_{corrected}$ in the target vessel (R= 0.34 and P<0.05) There is a trend towards a correlation between number of stents used and degree of change in $IMR_{corrected}$. We also see a negative correlation between the diameter of vessel undergoing PCI and change in reference vessel IMR (R= –0.39 P<0.05), suggesting that the greater the diameter of the target vessel treated, a greater reduction in IMR is seen in the reference vessel.

Table 4.8 Baseline characteristics of patients with increase of $IMR_{corrected}$ in the target vessel following PCI compared with reduction or no change in $IMR_{corrected}$. *Mean+/–SEM

	$IMR_{corrected}$ tar nochange/reduction (n=24)	**$IMR_{corrected}$ tar increase (n=11)**	
Age*	60.2+/–1.75	60.45+/–2.53	*P*=0.94
BMI*	29.6+/–0.96	30.35+/–1.28	*P*=0.65
Hypercholesteraemia	95.8% (23)	90.9% (10)	*P*=0.99
Hypertension	70.8% (17)	54.5% (6)	*P*=0.34
Diabetes	25% (6)	18.2% (2)	*P*=0.65
Smoking history in 12 months before PCI	45.8% (11)	45.5% (5)	*P*=0.99
Family history CAD	66.7% (16)	63.6% (7)	*P*=0.86
CCS class I	12.5%(3)	9.1% (1)	*P*=0.49
CCS class II	54.2%(13)	36.4%(4)	*P*=0.49
CCS class III	33.3%(8)	54.5%(6)	*P*=0.49
Aspirin(n)	95.8% (23)	100% (11)	*P*=0.67
Beta-blockers(n)	79.2% (19)	72.8% (8)	*P*=0.67
Ca antagonist(n)	45.8% (11)	63.6% (7)	*P*=0.86
Nitrates(n)	33.3% (8)	36.4% (4)	*P*= 0.33
Nicorandil(n)	29.2% (7)	18.2% (2)	*P*=0.49

ACE-I (n)	45.8% (11)	45.5% (5)	*P*=0.98
Bare metal stent (n)	50% (12)	45.5% (5)	*P*=0.80
Drug eluting stent (n)	50% (12)	55.5% (6)	*P*=0.80
LDL, mmol/l*	1.82 (+/–0.12)	2.06 (+/–0.19)	*P*=0.73

There is no difference in baseline characteristics between the patients who developed an increase in IMR post-PCI compared with those that had either a reduction or no overall change as shown in Figure 4.8.

Table 4.9 Comparison of haemodynamic characteristics of patients with increase of $IMR_{corrected}$ in the target vessel following PCI compared with reduction or no change in $IMR_{corrected}$. *P<0.05, **P<0.01

	$IMR_{corrected}$ target nochange/reduction (n=24)	**$IMR_{corrected}$ target increase (n=11)**	
CFR ref pre	1.70+/–0.15	2.54+/–0.58	*P*=0.07
CFR tar pre	3.08+/–0.35	2.74+/–0.48	*P*=0.59
FFR tar pre	0.62+/–0.02	0.69+/–0.04	*P*=0.17
CFI	0.21 (0.15–0.27)	0.21 (0.15–0.37)	*P*=0.74
IMR ref pre	26.2 (17.0-34.1)	23.0 (13.4-32.0)	*P*=0.33
$IMR_{uncorrected}$ tar pre	**25.7 (17.0-27.2)***	**12.9 (10.1-28.6)**	***P*=0.03**
$IMR_{corrected}$ tar pre	**17.3 (13.6-27.2)****	**8.2 (5.6-13.6)**	***P*=0.002**
$IMR_{resting}$ ref rest pre	83.9 (60.3-103.9)	68.3 (33.5-100.7)	*P*=0.25

$IMR_{uncorrected\ resting}$ tar pre	47.1 (39.1-58.2)	33.1 (19.3-67.8)	*P*=0.85
$IMR_{corrected\ resting}$ tar pre	33.8 (29.1-51.2)	29.1 (15.2-67.7)	*P*=0.79
Periprocedural myocardial injury	25% (7)	27.3%(9)	*P*=0.62

CFI, collateral flow index; IMR, Index microcirculatory resistance; ref= reference vessel; tar, target vessel; CFR, coronary flow reserve; FFR, fractional flow reserve.

We find a marked reduction in IMR in the target vessel pre-PCI (both uncorrected and corrected respectively) in the group of patients with an increase of IMR when compared with either no change or a decrease of IMR (25.7[17.0-27.2] vs. 12.9[10.1-28.6] *P*<0.05) (17.3[13.6-27.2] vs. 8.2[5.6-13.6] *P*<0.01) respectively. There appears to be a trend of impaired CFR in the reference vessel in the group which have no change or decrease of IMR although this is just below the level of significance (1.7+/–0.15 vs. 2.54+/–0.58 *P*=0.07).

4.4 Discussion

The results obtained during this study shows the effect of PCI on microvascular function immediately following the procedure in stable angina. Our findings are consistent with previous data obtained by other studies in the target vessel when collateral flow is not taken into account. In addition to this however, we obtained novel data on the target vessel corrected MR and the reference vessel which has not, to our knowledge, been previously reported.

We find a heterogenous response in target vessel $IMR_{corrected}$. The only procedural factor associated with an increase of IMR is number of inflations. The only haemodynamic predictor of increase of IMR in the target vessel is a low pre-PCI $IMR_{corrected}$ in the target vessel. We also found $IMR_{uncorrected}$ overestimates pre-PCI MR significantly, in some cases we found that P_w is nearly as high as P_d, the greater the P_w the greater the overestimation of $IMR_{uncorrected}$. We found a reduction in the reference vessel microvascular resistance in response to PCI in the target vessel, which is a novel finding. $IMR_{corrected}$ resting increases in the target and goes down in the reference vessel following PCI. The underlying mechanisms for microvascular dysfunction in target vessel and an apparent improvement in MR in the reference vessel requires further investigation.

This is the first study to provide a detailed invasive assessment of the microcirculation in both vessels taking into account the role of collateral circulation using the thermodilution method. We found a heterogenous response in IMR in the target vessel and no overall change when collateral flow is taken into account and a reduction in target vessel IMR when collateral flow is ignored. In addition we found a reduction in IMR in the reference vessel territory. There is a debate regarding best measurement of MR (see chapter 2 page 48). CFR has its limitations due to dependency on basal conditions and haemodynamic factors. A few studies have used a Doppler derived minimal MR_v, yet although this resolves issues with variability in basal flow it does not account for collateral flow. As a result significant reductions in MR following PCI

were found in the early Doppler studies. In our study $IMR_{uncorrected}$ is equivalent to MR_v hyperaemic in Doppler studies. $IMR_{corrected}$ is the only method of microvascular assessment which is not only independent of haemodynamic factors, but also corrects for collateral flow.

The microvascular response to PCI measured in terms of actual microvascular resistance has only been studied by a small number of investigators. The earlier studies used a combination of Doppler catheter to measure coronary artery velocity proximal to the stenosis, a pressure wire to measure distal coronary pressure and QCA to measure the cross sectional area of the vessel. These studies found a trend in reduction in MR_v however they failed to reach statistical significance (Sambuceti *et al.*, 2001;Marzilli *et al.*, 2002). They may have overestimated the flow in the vessels since they measured velocity proximal to the lesion in the pre stenotic segment with the Doppler catheter and we know that the velocity distal to lesion can be reduced.

We see a reduction in $IMR_{uncorrected}$ in response to PCI. We may expect to see a reduction in the target vessel since pressure is increased and hence flow will increase and resistance will be less, however this is only true if the contribution of collateral flow is not taken into account. The improvement in $IMR_{uncorrected}$ post-PCI is similar to data from Doppler studies which have calculated minimal MR based on distal pressure and velocity, and not accounted for collateral flow (Chamuleau *et al.*, 2003;Siebes *et al.*, 2004). Verhoeff *et al.* have confirmed similar findings of a reduction in IMR

following PCI, to the extent that MR_v post-PCI is actually less than MR_v in reference vessel (Verhoeff *et al.*, 2005). We see a comparable degree of reduction in $IMR_{uncorrected}$, overall there was a 40% reduction in $IMR_{uncorrected}$ following PCI. We also found a correlation between FFR pre and degree of change in $IMR_{uncorrected}$ R= 0.51 P<0.01, yet there was no similar relationship when reference vessel IMR and target vessel $IMR_{corrected}$ were compared (reference R=0.27 P=0.14 target corrected R= 0.23 P=0.19). This would suggest those subjects with more severe stenosis have more reduction in microvascular resistance following PCI if $IMR_{uncorrected}$ is used. One would assume this was because more severe stenosis will have greater collateralisation and hence pre-PCI $IMR_{uncorrected}$ would be overestimated in those with the more severe the stenosis. We tested this by comparing the association between P_w and FFR target vessel pre-PCI. We find a significant inverse association between P_w and FFR pre-PCI in the target vessel (R= –0.44 P<0.01). This finding supports the notion that greater degrees of collateralisation are found in patients with greater severity of epicardial stenosis. Previous data has shown that coronary artery lesion severity is an independent predictor of the degree of collateralisation (Pohl *et al.*, 2001). There are significant differences in interpretation depending on which method is utilised to assess IMR particularly when epicardial stenosis is present.

The inclusion of the collateral component of flow is essential in our study population since we have a population of haemodynamically significant single vessel disease as seen by a mean FFR of 0.61 in our study population. Thus we have included

$IMR_{corrected}$ as the main marker of microvascular function in the target vessel. The greater collateral contribution the greater the over estimation of MR, therefore in these patients pre-PCI IMR will be overestimated, due to this it seems apparent that IMR actually decreases post-PCI, but actually when we observe changes in $IMR_{corrected}$, we see that although the response is heterogenous and IMR goes up as well as down in different patients, overall as a group, there is no change in IMR in response to PCI (Figure 4.5). Our data are similar to recent findings by Fearon's group, which also found overall no change in $IMR_{corrected}$ following PCI to the LAD in patients with stable angina (Ho *et al.*, 2010).

The heterogenous response of IMR following PCI implies differing effects on MR in different patients. Of particular interest is the group of patients that have an increase in microvascular resistance following PCI, since these are the ones associated with poor outcome in previous studies when CFR was used a the measure of microvascular function. A third of our study population develop an increase of $IMR_{corrected}$, the underlying cause of increased IMR post-PCI is still unclear, although there are many hypothesis for potential mechanisms. Traditionally one of the key mechanisms suggested for periprocedural microvascular injury is that of distal embolisation (DE) of plaque and thrombus material following PCI (Kotani *et al.*, 2002). Despite the fact that distal protection devices have been shown to eliminate evidence of DE as detected by high intensity signals with a Doppler wire (Okamura *et al.*, 2005), there is no transferred beneficial effect on microvascular integrity and cardiac events. This raises

questions as to the role of DE in microvascular dysfunction in native vessels. In addition we see no relationship between periprocedural myocardial necrosis and change in IMR (data presented in chapter 5), suggesting that mechanisms separate to myocardial necrosis may be responsible for microvascular dysfunction.

From our data so far, we have established the only haemodynamic factor which seems to be predictive of increase in IMR in the target vessel is the IMR pre-PCI in the target vessel. Very low baseline IMR is associated with an increase of IMR. This may be explained by the fact that very low IMR is found in certain patients due to prolonged ischaemia, but following successful PCI, to prevent sudden increase in pressure and therefore potential microvascular injury, a vasoprotective mechanism could be responsible for an increase in IMR. This mechanism could be a myogenic reflex, which is vasoconstriction in response to increases in pressure to regulate flow and prevent microvascular damage by sudden upsurge in pressure. However, this mechanism may appear to be in contrast to findings by Marzilli *et al.* Contrary to popular opinion, they found resting microvascular vasoconstriction in response to ischaemia rather than vasodilation, this was reversed with adenosine (Marzilli *et al.*, 2000). It should be noted the study by Marzilli *et al.* assessed resting MR, we find a low hyperaemic MR is associated with an increase in IMR.

The only procedural factor which is associated with upregulation of IMR is the total duration of balloon inflation. These findings are similar to this study by Cuisset *et al*

(Cuisset *et al.*, 2008). They found direct stenting had better post-PCI IMR compared with predilation and stenting. This would support the original notion that it is the repeated cessation and restoration of flow which allows for a greater interaction of inflammatory cells with their respective cellular adhesion molecules, and this creates conditions in which microvascular dysfunction is promoted. However, it must be noted that this could as well be a confounding factor in as much as more difficult procedures will need more inflations, and hence greater risk of microvascular injury.

Our data show that the mean FFR increases from 0.6 to 0.91 post-PCI in the target vessel. This reflects firstly the haemodynamic significance of lesions in our study; FFR less than 0.75 has been accepted as a reliable indicator of haemodynamic significance of the lesion and revascularisation should be performed (Pijls *et al.*, 2007). Secondly the improvement in FFR to above 0.9 indicates a successful procedure in terms of epicardial revascularisation particularly since FFR greater than 0.9 equates with good medium term outcome (Pijls *et al.*, 2002b). In addition, FFR in the reference vessel is 0.95 thereby demonstrating that we have a sample in which the reference vessel is haemodynamically normal. We could not always achieve a post-PCI FFR >0.9 post-PCI in the target vessel. In these cases a hyperaemic pullback measurement of FFR was performed. Suboptimal FFR was due to diffuse coronary artery disease in the vessel as seen by a gradual increase in FFR as the pullback was performed. The FAME study has recently confirmed the efficacy of using FFR as a tool to guide decision making and has shown that death, MI and repeat

revascularisation are reduced in patients with therapy guided by FFR (Tonino *et al.*, 2009).

The earlier studies which measured CFR before and after PCI suggested impaired CFR was due to a combination of an increase in baseline flow, diffuse atherosclerosis and a more generalised microvascular dysfunction, causing reduced CFR. In some patients following PCI there was a deterioration in microvascular function, with potentially severe consequences, where no obvious underlying cause can be found. It is this subtle form of microvascular injury which is of particular interest. Although the limitations of CFR are well documented we have included CFR in our analysis since it is a useful marker for comparison with earlier studies.

We found that in target vessel there is an overall increase in CFR which would be expected, since CFR is a combined measure of epicardial and microvascular resistance (see Chapter 2 Figure 2.1). PCI relieves the epicardial stenosis and hence epicardial resistance is reduced. Earlier investigators have pointed out abnormal CFR in the reference vessel pre-PCI as a marker of a more generalised microvascular dysfunction, and if this is the case then the chance of successful microvascular reperfusion and normalisation of CFR in the target vessel is less (Kern *et al.*, 1999) However, this could also have been due to increase in basal flow due to supply of resting collateral flow, therefore resting flow would need to be higher than if no stenosis was present in an adjacent vessel.

It is well established that CFR is dependent on the effect of baseline flow, hyperaemic flow and any residual epicardial resistance. In our data both resting and hyperaemic flow increase following PCI, yet the degrees of improvement in each (resting and hyperaemic) will determine CFR. In the target vessel there is more of an increase in hyperaemic flow than in baseline flow following PCI, whereas in the reference vessel there is more of increase in basal flow compared with hyperaemic flow, this could explain the trend towards reduction in CFR in the reference vessel.

The effect of PCI on CFR in the target vessel has earlier been discussed earlier but there is sparse and conflicting data on the effect of PCI on the reference vessel. Zijlstra found no change in reference vessel microvascular function post-PCI for single vessel CAD, however they only had 12 patients in the reference vessel arm (Zijlstra *et al.*, 1988). Foley found no change in reference vessel CFR in response to PCI in the target vessel but again there were only 10 patients in the study (Foley *et al.*, 1995). A few studies have assumed pre-PCI CFR in the reference vessel will be the same as CFR post-PCI (Kern *et al.*, 1999), and this assumption was used to measure relative CFR, which was a predecessor to FFR as a means of assessing epicardial lesion severity. This assumption can lead to inaccuracies since microvascular function in the reference vessel can be dynamic following PCI rather than fixed, as we have demonstrated here.

Another small study showed improvement in CFR in reference vessel following PCI when there were visible collaterals, however the increase in CFR was due to a reduction in resting flow post-PCI rather than an increase in hyperaemic flow (Kyriakides *et al.*, 1998). CFR immediately post-PCI was found to be normal in the reference vessel of patients undergoing PCI of a target vessel however they did not measure CFR immediately pre-PCI to see how CFR changes with PCI (van Liebergen *et al.*, 1998).

These data conflict with our own: firstly we see an increase in resting flow post-PCI in the reference vessel, but we also see an increase in hyperaemic flow as well, but the increase in resting flow is greater than increase in hyperaemic flow, such that the CFR post-PCI in the reference vessel in our study has a downward trend. The most interesting haemodynamic finding in this study is the novel reduction in IMR of the reference vessel, we observe a 25% reduction of hyperaemic IMR in reference vessel. The reduction of IMR in the reference vessel could simply be explained by changes in arterial pressure following PCI, however we have ruled this out, since Pd does not change in the reference vessel post-PCI. All of the reduction in IMR is due a reduction in T_{mn}, and thus due to an increase in hyperaemic flow. There are sparse data on the effect of PCI on the reference vessel, and many of the studies which have investigated adjacent vessel bed flow have used CFR as a marker of microvascular function (Zijlstra *et al.*, 1988;Foley *et al.*, 1995;Kyriakides *et al.*, 1998;Pacella and Villanueva, 2006). There is only one previous study which has measured IMR in the reference

vessel following PCI in the target vessel (HOOLE *et al.*, 2010). The results from this small study contradicts our own, they found the reference vessel IMR increased following PCI, however most of the reference vessels in this study had haemodynamically significant lesions. The investigators did not measure coronary wedge pressure, which is will be important for assessment of IMR if significant epicardial stenosis is present as IMR will be overestimated as discussed earlier. In view of these disparities comparing our findings with their study has to be done with caution.

There is further evidence of changes in adjacent vessel haemodynamics as shown by a myocardial contrast echo model of induced epicardial stenosis in the LAD of canines. This showed presence of a stenosis reduced hyperaemic blood flow and increased resting blood flow in the circumflex territory when compared to the same vessel before stenosis was created. It was suggested the underlying mechanism may be related to recruitment of collateral microvessels allowing diversion of blood supply to the stenotic bed (Pacella and Villanueva, 2006). Although our study is in some ways reverse version of Pacella's, we have some similar findings. Since we relieve obstruction in one territory and microvascular function improves in the adjacent bed and hyperaemic flow improves, whilst they create a stenosis in the LAD causing resting flow to increase in the adjacent epicardial vessel and hyperaemic epicardial and microvascular flow to reduce in the adjacent vessel bed with an increase in MR in the adjacent vessel territory. It must be noted however that the stenosis in this experiment

was created acutely, however in patients with chronic stenosis there is evidence of microvascular remodelling to compensate for chronic low distal pressure, and hence distal microvasculature may behave differently when the stenosis is relieved (Bakker *et al.*, 2002).

There is evidence of impaired coronary flow in patients with single vessel disease in an adjacent normal vessel (Sambuceti *et al.*, 1993;Uren *et al.*, 1994). The underlying mechanism responsible for a reduction in hyperaemic and basal IMR in the reference vessel may be related to collateral flow from the donor vessel, yet we found no relationship between CFI and baseline IMR and degree of change in IMR in the reference vessel. If collateral flow is not a significant factor in the reduction of IMR in the reference vessel, then it points to another mechanism causing further vasodilation of the microcirculation over and above that of adenosine and intracoronary nitrates. It seems plausible that either a mechanical pathway is activated which responds to increase in stretch in the epicardial vessel with a vasodilatory effect in the reference vessel territory, for example a neurally mediated reflex, yet if this was the case we would expect an increase in IMR in the reference vessel (Gregorini *et al.*, 1994), since balloon dilation of the target vessel may cause activation of stretch receptors in the collateral bed leading to vasoconstriction and hence increase in IMR. When intracoronary nitrates are administered and adenosine then there are only a few agents which can cause further vasodilation, such as yohimbe, urapadil or phentolamine (alpha adrenoreceptors antagonists) (Barbato *et al.*, 2004b). Alpha adrenoreceptors are

important in maintaining epicardial and microvascular tone, alpha-1 adrenoreceptors predominate at epicardial level and alpha-2 adrenoreceptors at the microcirculatory level. It is well known that PCI can activate both alpha-1 and alpha-2 adrenoreceptors causing epicardial and microvascular vasoconstriction (Gregorini *et al.*, 2002). We may expect to see vasoconstriction in the adjacent vessel territory in response to stretching the target vessel however we observe the reverse of this. To our knowledge the action of adrenoreceptors activity on the reference vessel territory has not been investigated. Other possibilities to explain the reduction in IMR in the reference vessel include release of vasoactive mediators in the target vessel territory or via changes in collateral flow. Our findings regarding the changes seen in the reference vessel are worthy of further investigation to understand better the complex interactions between microcirculation in adjacent vessel beds.

We found an inverse association between $IMR_{corrected}$ in the target vessel pre-PCI and CFI ($R= -0.36$ $P<0.05$), there was no association between IMR in the reference vessel and CFI. Other studies have shown CFR in the target vessel is related to adjacent vessel resistance and inversely related to collateral resistance (Billinger *et al.*, 2001). Our data would suggest the greater the CFI the lower the IMR in the target vessel. These findings are suggestive of collateral vessels playing an important role in the regulation of microvascular function between different vessel beds. Therefore we chose to analyse collateral flow by comparing patients with good collaterals vs. poor collaterals. We find there is a trend towards a worse CFR pre-PCI in the target vessel

with high CFI compared with low CFI (1.61+/–0.16 vs. 2.39+/–0.42) respectively, yet this is not statistically significant and these are similar findings to the study by Billinger *et al.* This could be explained by greater basal flow supplying the target vessel territory in the good collateral group.

We find significantly lower microvascular resistance in the reference vessel pre and post-PCI in the high CFI group compared with low CFI. In the target vessel although there are trends of reduced $IMR_{corrected}$ and $IMR_{uncorrected}$ in the good collateral group, this does not reach significance. The lower microvascular resistance in the reference vessel may be explained by the need for a pressure gradient from high to low to maintain collateral flow from the reference vessel to the target vessel territory in those subjects with good collateralisation. The lower IMR in the reference vessel in patients with better collaterals is a novel finding, most of the data on collateral function has been on patients with CTO (Zimarino *et al.*, 2006;Werner *et al.*, 2006a). The physiological response of collateral flow and microvascular function cannot be assumed to be the same in patients with complete occlusion of the vessel and those with patent anterograde flow. What we see in the good collateral group may reflect a microvascular system which is better functioning and more adaptive to acute changes in coronary physiology.

We have also assessed the differences in basal microvascular resistance in response to PCI, we find that both resting and hyperaemic IMR is reduced following PCI in the

reference vessel. $IMR_{corrected}$ resting increases in the target vessel and there is a reduction in $IMR_{uncorrected}$ hyperaemic in the target vessel. Resting IMR may be useful since it gives an indication to the basal tone of the microcirculation in response to PCI, thus more reflective of the "real-life" clinical scenario. However, resting IMR has the same limitations as CFR since both are dependent on basal haemodynamic conditions which are variable. There are sparse data on basal microvascular tone in vivo models in response to PCI. A non significant increase in basal tone was seen by Sambuceti *et al.* following PCI (Sambuceti *et al.*, 2001). Verhoeff found no effect of PCI on basal microvascular tone (Verhoeff *et al.*, 2005). However once again collateral flow was not accounted for, which may explain why they found no change in resting MR. When we calculated $IMR_{uncorrected}$ resting we see no overall significant change, although there is trend towards increase in IMR which is similar to data from Sambuceti. There is a large increase in resting $IMR_{corrected}$ in response to PCI in the target vessel which implies a marked increase in basal tone, thereby pointing towards vasoconstriction in the microcirculation in the "real life" post-PCI scenario. When adenosine is given, this increase in basal tone is reversed. This may explain why adenosine can be a useful adjunct in no-reflow in certain patients following PCI (Mahaffey *et al.*, 1999;Vijayalakshmi *et al.*, 2006). The underlying mechanism responsible for the increase in basal resistance post-PCI may be due to autoregulatory microvascular control to prevent microvascular damage and if this is the case, may be mediated by the myogenic pathway. It is thought that a rise in intraluminal pressure activates stretch receptors on the surface of the smooth muscle cell, causing cation influx and

subsequent depolarisation of the smooth muscle cell activating the voltage gated calcium channel (L type), allowing for calcium influx into the cell (Nelson *et al.*, 1990;Davis *et al.*, 1992). When we assessed the relationship between change in Pd resting post-PCI vs. change in resting $IMR_{corrected}$ we fail to see any relationship (r= – 0.26 P=0.13). This finding does not necessarily rule out the myogenic response as a mediator, but it is more likely that there are several factors responsible, since the regulation of the microcirculation is a delicate balance between several different mechanism including, flow mediated dilation, myogenic, metabolic, neurally mediated, and as well as physical forces such as left ventricular end diastolic pressure and left ventricular wall tension.

4.5 **Limitations**

We acknowledge there some limitations to our experiment in measuring certain haemodynamic indices. We measured wedge pressure for CFI and $IMR_{corrected}$. It may be argued that the wedge pressure will change acutely post-PCI and hence inclusion of the first wedge pressure measured pre-PCI to correct for IMR post-PCI is not valid. Although this may be true in certain patients with CTO (Zimarino *et al.*, 2006), the evidence is equivocal. Perera found no change in CFI at 24 hours in patients with CTO (Perera *et al.*, 2007). Our data have shown there is no difference in coronary wedge pressure acutely following PCI, which is similar to other studies (Jensen *et al.*, 2007). In addition our study group consists of patients with stable angina rather than CTO, we

would expect more severe changes in collateral flow in CTO purely due to the much greater role collaterals play in this group of patients (Werner *et al.*, 2006b).

The IMR in the distal vessel may be overestimated particularly if the increase in flow and pressure causes passive distension of the vessel. Since the thermodilution method assumes that the volume between the proximal shaft and distal sensor remain unchanged, if volume was to increase then true MR would be less than calculated if volume truly remained unchanged. It should also be noted that we may have introduced some bias into the data because we were unable to record pre-PCI data in the target vessel in three patients with very tight stenosis due to unrecordable transit time.

The measurement of IMR is dependent on the distance between the distal sensor in the coronary artery and the tip of the guide catheter. If there is a difference in this distance between patients, differing values of IMR will be calculated. We were unable to ensure that this distance was equal in all patients due to anatomical variations, but we did however ensure the wire was as distal as possible in all vessels. This limitation has to be taken into consideration when the data is pooled and is particularly problematic if we are comparing individual inter-patient differences in IMR, although we have not analysed data in this way. However, since the majority of our data is comparing intra-patient changes in IMR, this limitation has little effect on the overall results.

CFI was calculated by estimating venous pressure (P_v) to be 5mmHg since we did not measure P_v for our study. Although the addition of P_v will provide a more accurate CFI for the purpose or our study, it has been shown that CFI can be overestimated if P_v is estimated rather than measured and a small percentage of subjects may be misclassified (Perera *et al.*, 2004). In our data the margin of error will be small by failing to measure P_v making it unlikely to have a significant impact on the outcome of our data. In addition, given that P_v does not change acutely following PCI (de Marchi *et al.*, 2005), then failure to assess P_v for the purpose of IMR should make little difference, since we are comparing changes in IMR in the same patient and same vessel.

Ideally central administration of adenosine is recommended for haemodynamic measurements, however in our study group, since we are comparing like for like before and after PCI, overall changes will be negligible. In addition there is emerging evidence of little difference between central and peripheral administration of adenosine (Seo *et al.*, 2010). We did not measure wedge pressure in the reference vessel for obvious reasons such as the risk of arterial injury, endothelial dysfunction, dissection and development of atheroma in normal vessels. We assume that collateral flow is negligible and close to zero, hence in the reference vessel it is justified to use the IMR uncorrected equation.

In summary therefore, the results obtained during these studies provided baseline data on the natural history of microvascular function in response to PCI which was consistent with previous data obtained by other workers in the target vessel when collateral flow not taken into account. In addition to this however, we obtained novel data on the target vessel corrected MR and reference vessel data which has not, to our knowledge, been previously reported.

This is the first study to use IMR as a measure of MR and assess the effect of PCI on the microcirculation. We find a heterogenous response in target vessel $IMR_{corrected}$. The only procedural factor associated with upregulation of IMR is the number of inflations. The only haemodynamic predictor of upregulation of IMR is a low pre-PCI $IMR_{corrected}$ in the target vessel. We found $IMR_{uncorrected}$ overestimates pre-PCI MR significantly, in some cases we found that P_w is nearly as high as P_d, the greater the P_w the greater the overestimation of $IMR_{uncorrected}$. The reduction in the reference vessel microvascular resistance in response to PCI in the target vessel is a novel finding. The underlying mechanisms for microvascular dysfunction in target vessel and an apparent improvement in MR in the reference vessel requires further investigation.

Chapter 5 Inflammation

5.1 Introduction

A large number of studies have identified the crucial role of inflammation in the pathogenesis of atherosclerosis from the very earliest detectable phase to the development of plaque rupture resulting in acute coronary syndromes (Ross, 1999b;Libby and Ridker, 2006). Increased levels of various circulating inflammatory markers predict an increased risk of vascular events such as MI, stroke, peripheral vascular disease and sudden death in previously healthy populations (Ridker *et al.*, 1997;Ridker *et al.*, 2000a). Patients with ACS have higher levels of baseline CRP compared to stable angina (Berk *et al.*, 1990); (Cusack *et al.*, 2002). In addition it was noted that increased levels of inflammation in patients diagnosed with acute coronary syndrome or acute MI confers a worse prognosis (Liuzzo *et al.*, 1994). Morrow *et al.* identified increased CRP as an powerful predictor of early mortality in 541 patients with acute coronary syndrome when combined with circulating troponin-T levels, moreover CRP remained a strong predictor of mortality independent of troponin-T (Morrow *et al.*, 1998). The CAPTURE study identified elevated baseline CRP as a strong independent predictor of MI and death six months following PCI (Heeschen *et al.*, 2000).

Similarly many studies have shown that increased basal levels of inflammation before PCI are associated with a worse outcome. Buffon *et al.* first described the relationship

between increased baseline levels of inflammation and increased early and late complications in patients undergoing PCI (Buffon *et al.*, 1999). They found that inflammatory activation is even more a predictor of complications than the clinical presentation of unstable angina. A small study of 62 patients undergoing PCI for unstable angina found those with normal CRP pre-procedure have excellent 12 month prognosis, whereas only 40% of those with raised CRP pre-PCI were event-free at 12 months (Versaci *et al.*, 2000). The first large study to evaluate the significance of elevated basal inflammation on outcome following PCI was performed by Chew *et al.* They studied over 700 patients undergoing PCI for stable or unstable angina and found those patients in the highest quartile for baseline CRP had over 3.5 fold increase in 30-day death or MI compared with those in lowest quartile (Chew *et al.*, 2001b). A similar increase in mortality and MI was detected at 2-year follow up in patients with elevated baseline inflammation (de Winter *et al.*, 2002).

PCI causes trauma to the endothelium of the coronary artery due to balloon inflation against the vessel wall, which also causes plaque rupture and triggers immune mediated mechanisms which include leukocyte, platelet and endothelial activation both locally and systemically (Inoue *et al.*, 1996;Serrano *et al.*, 1997). Inflammatory activation can be easily detected by a systemic rise in cytokines following PCI (Azar *et al.*, 1997). Higher levels of peak CRP post-infarction in patients with acute MI is associated with increased risk of heart failure and death, independent of conventional risk factors and independent of left ventricular dysfunction (Suleiman *et al.*, 2006).

Similar data suggests elevated CRP post-MI is a risk factor for death and heart failure, independent of troponin-T rise and traditional risk factors (Bursi *et al.*, 2007). An experimental MI study in rats has shown the addition of pure human CRP increases infarct size by 40% compared with controls (Griselli *et al.*, 1999). Furthermore the increase in infarct size was abolished by complement depletion with the addition of cobra venom factor, therefore suggesting a combination of CRP and activation of the complement system was responsible for further myocardial necrosis. Grisselli's study suggests the inflammatory response is more detrimental than levels of basal inflammation per se. These findings have been reflected clinically in patients undergoing PCI; many studies have found an association between adverse prognosis and increased inflammatory response following coronary angioplasty (Gottsauner-Wolf *et al.*, 2000;Saleh *et al.*, 2005;Gach *et al.*, 2007). Moreover sustained increase in inflammation following PCI is associated with worse outcome (Gaspardone *et al.*, 1998). These observations suggest inflammation is important not only in the pathogenesis of atherosclerosis and myocardial infarction but also in predicting complications from PCI. The inflammatory response to PCI seems to provide additional prognostic information yet the underlying mechanisms are poorly understood.

5.2 Justification of use of clinical markers

The markers we decided to use provide a measure of different aspects of the overall inflammatory process, endothelial activation, platelet activation and myocardial

necrosis. CRP is an acute phase protein produced by hepatocytes in response to inflammatory cytokines such as IL-6 and IL-1 (Moshage *et al.*, 1988). CRP is stable clinical marker stable with very little fluctuation except in case of acute phase response where there can be a massive increase in circulating levels, in fact the stability of CRP is similar to blood pressure measurements and LDL levels over time (Danesh *et al.*, 2000). It is also the most extensively studied inflammatory marker and there is a huge amount of data examining the role of CRP in coronary artery disease. IL-6 is a circulating cytokine which is secreted from a number of different cell types including lymphocytes and activated macrophages. IL-6 has been shown to be produced in the myocardial microcirculation, (Cusack *et al.*, 2002) and is known to be a major initiator of the acute phase response by stimulating production of CRP in the liver (Heinrich *et al.*, 1990). In addition, IL-6 has been shown to be independent predictor of the risk of developing IHD (Biasucci *et al.*, 1996;Harris *et al.*, 1999) (Rifai *et al.*, 1999). IL-6 is detectable as soon as 15 minutes following an inflammatory stimulus, hence it may be a useful marker to assess the extent of systemic inflammatory response immediately post-PCI, this is the main reason why it was included in this study. We measured markers of endothelial activation by measuring soluble ICAM-1 and VCAM-1. Both of these are transmembrane proteins from the immunoglobulin gene superfamily. ICAM-1 is expressed on endothelial cells, leukocytes and other cells such as smooth muscle cells and fibroblasts. ICAM-1 promotes leukocyte arrest and adhesion. VCAM-1 is also expressed by many different cell types but mainly on endothelial cells and is more specific for endothelial

activation than ICAM-1 (Ballantyne and Entman, 2002). Soluble ICAM-1 and VCAM-1 are accepted as a measures of endothelial activation, as these molecule are expressed by activated endothelium and then shed and are detectable in the circulation (Mizia-Stec *et al.*, 2002;Wu *et al.*, 2003). The degree of detectable circulating ICAM-1 is directly correlated with surface expression of ICAM-1 in human umbilical vein endothelial cells (Leeuwenberg *et al.*, 1992), and circulating VCAM-1 correlates well with expression of VCAM-1 mRNA in human atherosclerotic lesions (Nakai *et al.*, 1995). In patients with established CAD, elevated VCAM-1 is predictive of cardiovascular events (Blankenberg *et al.*, 2001) and plasma levels of VCAM-1 are associated with degree of atherosclerotic burden (van, I *et al.*, 2002). In view of this we chose to include ICAM-1 and V-CAM-1 as markers of endothelial activation which can be readily detected and are correlated with their expression on the endothelial surface. We chose soluble P-selectin as a marker of platelet activation, since it is accepted that the inflammatory response is not confined to leukocyte-endothelial interaction but also complex platelet, leukocyte and endothelial interaction. sP-selectin is platelet specific cell surface glycoprotein also know as CD62p. It is expressed following an inflammatory stimulus and is found predominantly on the platelet surface but is also present on the endothelium (Blann *et al.*, 2003). sP-selectin mediates leukocyte rolling and recruitment via sP-selectin glycoprotein ligand-1 found on most leukocytes and is also responsible in part for platelet to platelet aggregation (Merten and Thiagarajan, 2000). sP-selectin is easy to measure with ELISA and peripheral levels reflect intracoronary levels (Jaumdally *et al.*, 2007). We also

measured serum levels of endothelin-1(ET-1). Although ET-1 is potent vasoconstrictor, it is also a modulator of neutrophil and leukocyte function and stimulates adhesion molecule expression. Since underlying mechanisms responsible for microcirculatory dysfunction may be multifactorial, we have included ET-1 in our analysis. Endothelin-1 has been shown to be an important peptide in the regulation of coronary macro and microcirculation (MacCarthy *et al.*, 2001). There is evidence that ET-1 may be a significant factor in microcirculatory dysfunction following both PCI in stable angina and primary PCI (Niccoli *et al.*, 2006;Papadogeorgos *et al.*, 2009). We measured expression of neutrophil MAC-1 receptor CD11b/CD18, which is a beta 2 integrin and a key component facilitating adhesion of leukocytes to activated endothelium. The activation of leukocytes has been implicated in ischaemia reperfusion injury and inhibition of the MAC-1 receptor CD11b/CD18 has shown a reduction in myocardial injury in experimental models (Simpson *et al.*, 1988).

Whilst the inflammatory regulation is thought to be a homogenous phenomenon, the inflammatory response is in fact a sequence of interactions between humoral signalling molecules, cell surface receptors and endothelial adhesion molecules. We chose to measure each of these processes with our choice of inflammatory markers and included certain additional ones such as endothelin-1 and sP selectin, which we suspect may be important in the mechanism of microvascular dysfunction.

5.3 Methods

Patients with symptomatic single vessel CAD were prospectively recruited. All patients had undergone prior coronary angiography and were symptomatic with angina. The protocol was approved by the Liverpool Research Ethics Committee and all patients gave written informed consent. Subjects with recent ACS or MI within the previous three months, and with active inflammatory disease were excluded on account of potential effects on microvascular function and inflammatory status. Multivessel disease, previous transmural myocardial infarct in the territory of the vessel to be dilated and chronic total occlusion of the target vessel, previous CABG or PCI in target vessel, short left main coronary artery, separate origin of LAD and circumflex artery and patients with contraindication to adenosine were all excluded.

All subjects attended the Liverpool Heart and Chest Hospital on the morning of the angioplasty and had blood samples taken from a vein in the antecubetal fossa, 30mls of blood was collected and placed in tubes with either serum gel or EDTA as appropriate. All normal medications were continued and all patients were given 600 mg aspirin and 600mg clopidogrel at least 12 hours before PCI. Coronary angioplasty was carried out in the standard manner and measurements of IMR_{hyp}, IMR_{res}, FFR and CFR before and after PCI, as described in Chapter 2. All subjects had repeat blood samples taken one hour post angioplasty and 24 hours later. Serum blood samples were immediately spun down at 3000rpm and transferred into labelled tubes and stored at –80°C until analysis. EDTA samples were analysed as described below.

5.3.1 ELISA

Levels of CRP, IL-6, sICAM-1, sVCAM-1, sP-selectin and ET-1 were measured using commercially-available ELISA assay kits (R&D Systems Europe, Ltd. 19 Barton Lane, Abingdon Science Park,. Abingdon, OX14 3NB). Samples were analysed in duplicate and the analysis was performed in the research laboratory at the LHCH.

5.3.2 Flow cytometry

Blood samples were collected into tubes containing liquid potassium EDTA (Becton Dickinson; 0.25% final concentration), mixed gently and kept at room temperature before analysis as described in chapter within 30 minutes. In separate tubes, 50µl of whole blood was added to 5µl of FITC-labelled CD45 and 5µl of pycoerythrin (PE)-labelled mouse anti-CD11b or PE labelled mouse immunoglobulin isotype control (BD Pharmingen). Cell preparations were incubated for 1 hour with gentle mixing at 4°C followed by erythrocyte lysis and cell fixation by incubating for 10 minutes at room temperatures with equal volumes of optilyse B (Becton Coulter Ltd) followed by the addition of 500µl of distilled water and further incubation for 10 minutes at room temperature.

All flow cytometric measurements were performed using Becton Dickinson FACScalibur, flow cytometer. Day to day alignment and calibration of this instrument

was achieved with commercial preparations of fluorescent micro-spheres (Coulter electronics Ltd, Luton, Bedfordshire, UK).

Leukocyte populations were isolated by CD45 fluorescence and forward and side scatter dot plots. The mean fluorescence intensity (MFI) of 10,000 cells was measured and compared to that of control cells incubated with matched PE-labelled isotope controls.

5.3.3 Statistical analysis

Data was analysed for normality and reported as mean +/– SEM (95% CI) or median and inter-quartile range as appropriate. Comparisons between two groups were made with Students paired t-test or Wilcoxon rank test as appropriate. Correlation coefficients were calculated using Pearson's coefficient or Spearman's Rank as appropriate. To obtain normally distributed data IMR was naturally log transformed. Multivariate analysis of linear regression was performed using change in IMR as the dependent variable. Those variables in the univariate analysis with $P<0.25$ were included in the multivariate linear analysis using backward selection procedure. Data analysis was performed using SPSS statistical software version 17.0 (SPSS Inc. Chicago Illinois).

5.4 Results

5.4.1 The effect of PCI on circulating inflammatory markers, adhesion molecules and endothelin-1

The next set of data describes the detectable changes in the circulating blood of subjects in relation to PCI.

Figure 5.1 Effect of PCI on HsCRP levels pre, 1 hour post and 24 hours post-PCI. Mean and SEM displayed in Figure. n=39 $**P<0.01$

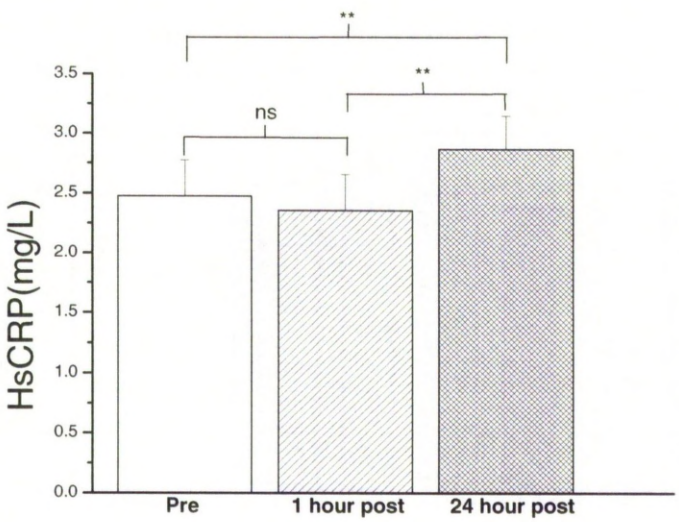


Figure 5.1 shows that there was no significant difference in HsCRP levels 1 hour post-PCI when compared with pre-PCI levels (2.47+/–0.34 mg/l vs. 2.36+/–0.3 mg/l $P=0.14$). There is a significant increase in HsCRP 24 hours post-PCI compared with pre-PCI levels (2.47+/–0.34 mg/l vs. 2.86+/–0.28 mg/l $P<0.01$), and also an increase

in HsCRP between 1 hour post and 24 hour post levels (2.36+/–0.33 mg/l vs. 2.86+/–0.28 mg/l $P<0.01$).

Figure 5.2 Effect of PCI on IL-6 levels pre, 1 hour post and 24 hours post-PCI. Mean and SEM displayed in Figure. n=39 ***$P<0.001$

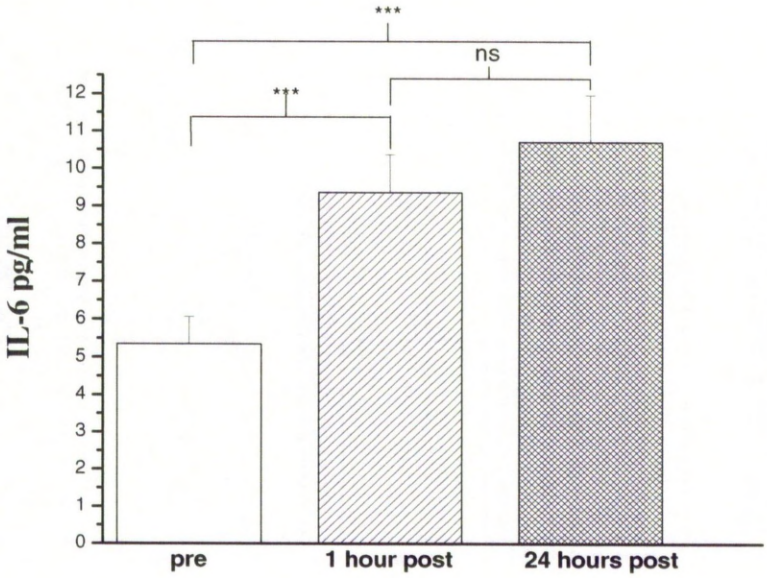


Figure 5.2 demonstrates a significant increase in systemic IL-6 levels one hour post-PCI (5.35+/–0.72 pg/ml vs. 9.36+/–0.99 pg/ml $P<0.001$), and 24 hours post-PCI (5.35+/–0.72 pg/ml vs.10.71+/–1.26 pg/ml $P<0.001$), when compared with pre levels. There was no significant difference between 1 hour post and 24 hours post levels of IL-6 (9.36+/–0.72 pg/ml vs. 10.71+/–1.26 pg/ml $P=0.63$).

Figure 5.3 Effect of PCI on soluble ICAM-1 levels pre, 1 hour post and 24 hours post-PCI. Mean and SEM displayed in Figure. n=39 $**P<0.01$

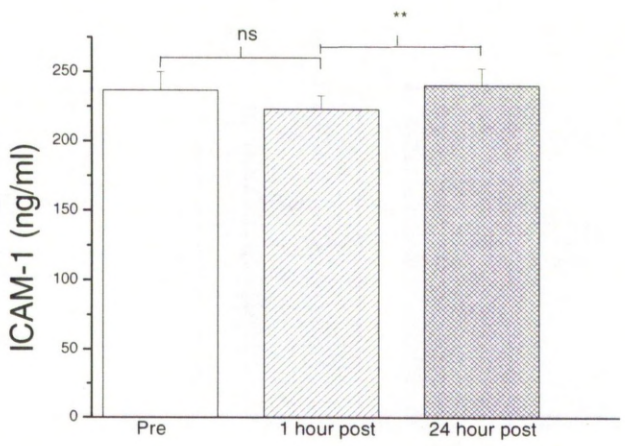


Figure 5.3 shows that we observed no significant change in soluble ICAM-1 1 hour post-PCI (236.8+/–13.1 ng/ml vs. 222.9+/–9.9 ng/ml $P= 0.06$). There was a small increase in soluble ICAM-1 between 1 hour post and 24 hour post samples (222.9+/–9.9 ng/ml vs. 240.3+/–12.3 ng/ml $P<0.01$). However overall between the pre and 24 post levels of soluble ICAM-1 there was no significant difference (236+/–13.12 ng/ml vs. 240.3+/–12.3 ng/ml $P=0.17$).

Figure 5.4 Effect of PCI on soluble VCAM-1 levels pre, 1 hour post and 24 hours post-PCI. Mean and SEM displayed in Figure. n=39 $**P<0.01$ $***P<0.001$

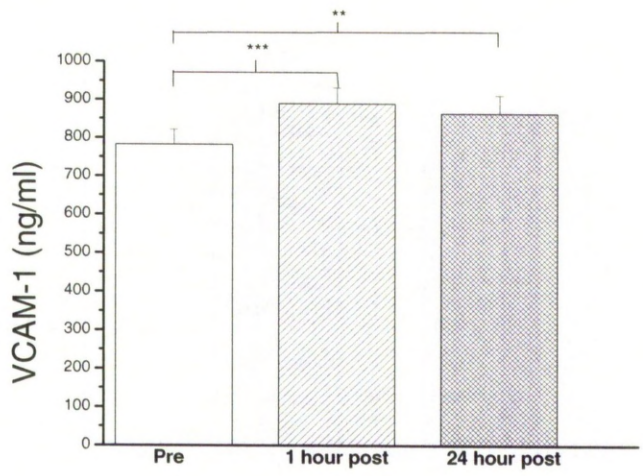


Figure 5.4 demonstrates an increase in soluble VCAM-1 1 hour post-PCI (782.5+/–39.6 ng/ml vs. 889.4+/–42.2 ng/ml $P<0.001$), there was no significant difference between 1 hour post and 24 hour post levels of VCAM-1 (889.4+/–42.2 ng/ml vs. 864.4+/–47.1 ng/ml $P=0.44$). The increase in VCAM-1 between pre and 24 post-PCI levels remains significant (782.5+/–39.6 ng/ml vs. 864.4+/–47.1 ng/ml $P<0.01$).

Figure 5.5 Effect of PCI on soluble P-selectin levels pre, 1 hour post and 24 hours post-PCI. Mean and SEM displayed in Figure. n=39 ***P<0.001

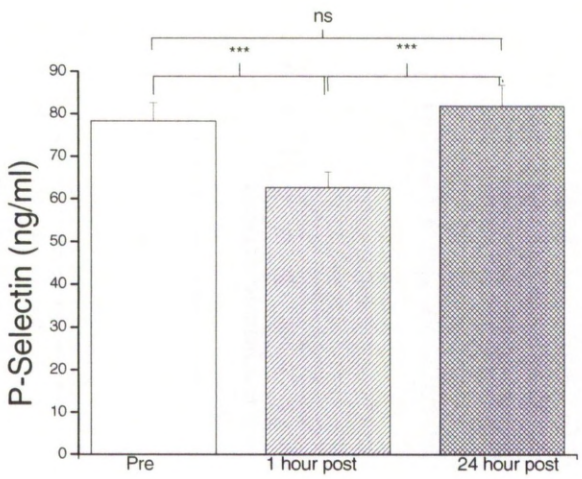


Figure 5.5 shows that we observed a decreased in soluble P-selectin 1 hour post-PCI (78.3+/–4.3 ng/ml vs. 62.7+/–3.6 ng/ml P<0.001). There was an increase in soluble P-selectin between 1 hour post and 24 hour post-PCI (62.7+/–3.6 ng/ml vs. 81.9+/–4.9 ng/ml P<0.001), however overall between pre and 24 hour post levels there was no significant difference (78.3+/–4.3 pg/ml vs. 81.9+/–4.9 ng/ml P=0.17).

Figure 5.6 Effect of PCI on endothelin-1 levels pre, 1 hour post and 24 hours post-PCI. Mean and SEM displayed in Figure. n=39 *P<0.05 **P<0.01

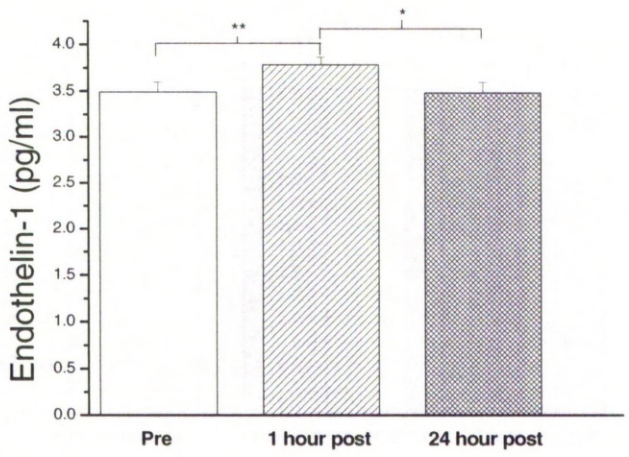
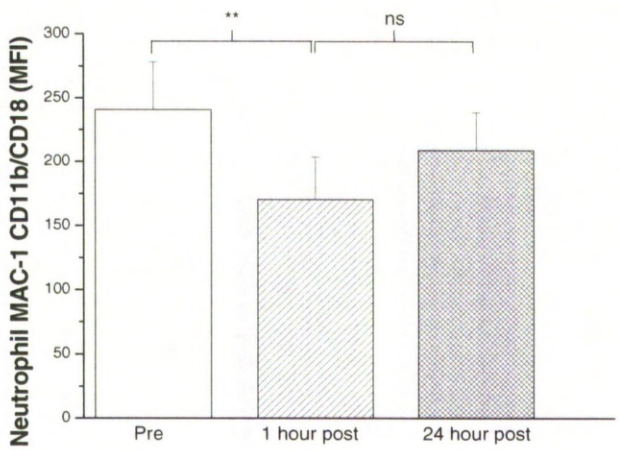


Figure 5.6 demonstrates an increase in endothelin-1 hour post-PCI (3.49+/–0.11 pg/ml vs. 3.78+/–0.08 pg/ml P<0.01). There was a mild reduction in endothelin-1 levels between 1 hour post and 24 hour post-PCI (3.78+/–0.08 pg/ml vs. 3.48 +/–0.11 pg/ml P<0.05) however overall between pre and 24 hour post levels there was no significant difference (3.49+/–0.11 pg/ml vs. 3.48+/–0.11 pg/ml P=0.97).

Figure 5.7 Effect of PCI on neutrophil MAC-1 receptor CD11b/CD18 expression pre, 1 hour post and 24 hours post-PCI. Mean and SEM displayed in Figure. n=34

$^{**}P<0.01$



We observed a reduction in MFI in neutrophil MAC-1 receptor CD11b/CD18 1 hour post-PCI (307.6+/–31.7 vs. 237.9+/–32.1 $P<0.01$), there was no difference between the 1 hour post and 24 hour post sample (237.9+/–32.1 vs. 265.7 +/–24.4 $P=0.17$) as shown in Figure 5.7. Of note however, there is no significant difference between pre and 24 hour post levels although there is a trend towards a reduction (307.6+/–31.7 vs. 265.7 +/–24.4 $P=0.14$).

In summary we have shown that there is a varied response to different inflammatory markers and mediators following PCI in stable angina patients. We see an increase in CRP, IL-6 and sVCAM-1 post-PCI, although this is most marked in IL-6. Overall at

24 hours there was no change in endothelin-1 and ICAM-1 and early reduction in sP-selectin and neutrophil MAC-1 receptor CD11b/CD18 which returns to pre-PCI levels at 24 hours.

5.4.2 Baseline inflammatory markers vs. pre PCI markers of microvascular function.

Given that we have demonstrated certain changes in inflammatory markers in our patient group, we analysed the relationship between inflammatory state and coronary microcirculatory dysfunction. This was performed by correlating basal levels of inflammation with markers of microcirculatory dysfunction pre-PCI.

Table 5.1 Relationship between baseline inflammatory markers and pre-PCI CFR in reference and target vessel. Pearson's correlation coefficient

Pre-PCI	**Reference**		**Target**	
	R (n=37)	***P***	**R (n=35)**	***P***
HsCRP	0.25	0.12	–0.18	0.31
IL-6	0.15	0.36	–0.05	0.78
ICAM-1	0.03	0.86	0.11	0.53
VCAM-1	0.28	0.09	0.05	0.77
ET-1	–0.01	0.93	–0.13	0.44
sP-selectin	–0.24	0.15	0.19	0.29
	Reference		**Target**	
	R (n= 30)	***P***	**R (n=28)**	**P**
CD11b/CD18	0.08	0.66	0.01	0.99

We see no relationship between baseline CFR and baseline inflammatory markers in both the reference and target vessels pre-PCI.

Table 5.2 Relationship between baseline inflammatory markers and pre-PCI IMR in the reference and target vessels. Spearman's rank coefficient

Pre PCI	IMR Reference R (n=37) *P*		$IMR_{corrected}$ Target R (n=35) *P*	
HsCRP	–0.42	0.80	0.18	0.29
IL-6	–0.03	0.87	0.06	0.72
ICAM-1	–0.01	0.41	–0.18	0.29
VCAM-1	0.03	0.88	–0.14	0.94
ET-1	0.17	0.31	0.32	0.07
sP-selectin	0.23	0.16	–0.02	0.92
	IMR Reference R (n=30) *P*		**$IMR_{corrected}$ Target R (n=28) P**	
CD11b/CD18	0.13	0.50	–0.07	0.71

There is no relationship between baseline inflammatory markers, adhesion molecules and endothelin-1 and pre-PCI microvascular resistance in either the target or reference vessel as shown in Table 5.2.

5.4.3 Basal inflammation vs. change in microvascular function following PCI as assessed by IMR and CFR.

We have assessed the relationship between basal levels of inflammation on the effect of PCI on microvascular function by correlating change in IMR and CFR against basal markers of inflammation, endothelial activation and platelet activation. Many of the earlier clinical studies suggest there may be an association between higher levels of basal inflammation and worse outcome following PCI (Buffon *et al.*, 1999;Saadeddin *et al.*, 2002;Iijima *et al.*, 2009), in addition this analysis is central to the main hypothesis of increased levels of basal inflammation predicting outcome of microvascular function.

Table 5.3 Relationship between baseline inflammatory markers and change in CFR expressed as CFRpost/CFRpre-PCI. Pearson's correlation coefficient. *$P<0.05$

Pre-PCI	**CFR Reference Post/pre PCI**		**CFR Target Post/pre PCI**	
	R (n=34)	***P***	**R (n=35)**	**P**
HsCRP	–0.04	0.81	–0.09	0.62
IL-6	0.01	0.97	–0.12	0.51
ICAM-1	–0.08	0.64	–0.23	0.18
VCAM-1	0.35	0.05	–0.17	0.33
ET-1	0.34	0.05	–0.02	0.93
sP-selectin	0.29	0.09	0.06	0.73
	Reference		**Target**	
	R (n=29)	***P***	**R (n=28)**	**P**
CD11b/CD18	0.08	0.61	–0.24	0.25

There are no associations between baseline inflammatory markers and change in CFR in the reference vessel.

Table 5.4 Relationship between baseline inflammatory markers and change in IMR expressed as IMR post/IMR pre-PCI. Spearman's rank coefficient. *$P<0.05$

Pre-PCI	IMR Reference Post/Pre PCI R (n =34)	*P*	$IMR_{corrected}$ Target Post/Pre PCI R (n=35)	*P*
HsCRP	0.18	0.32	–0.27	0.11
IL-6	0.02	0.89	–0.10	0.58
ICAM-1	0.35	0.05	0.24	0.16
VCAM-1	–0.06	0.74	0.18	0.32
ET-1	–0.20	0.25	–0.23	0.19
sP-selectin	–0.06	0.72	–0.15	0.37
	IMR Reference Post/Pre PCI R (n= 29)	***P***	**$IMR_{corrected}$ Target Post/Pre PCI R (n=28)**	***P***
CD11b/CD18	–0.32	0.09	0.17	0.40

There is no relationship between basal inflammatory markers and change in IMR in either vessel as shown in Table 5.4.

5.4.4 Comparison between the ratio of inflammatory response at 1 hour vs. change in microvascular function post/pre PCI

While there was little correlation between baseline levels of inflammatory markers and the change in IMR or CFR, given the evidence that the extent of the inflammatory

response may be important we looked at the magnitude of the response expressed as the ratio between pre and post levels (Saleh *et al.*, 2005;Gach *et al.*, 2007).

Table 5.5 Relationship between change in inflammatory markers at 1 hour post-PCI expressed as inflammatory marker 1 hour post-PCI/pre-PCI levels and change in CFR expressed as CFRpost/CFRpre-PCI. Pearson's correlation coefficient

Pre-PCI	**CFR Reference Post/pre PCI**		**CFR Target Post/pre PCI**	
	R (n=34)	***P***	**R (n=35)**	***P***
HsCRP	0.05	0.80	0.01	0.96
IL-6	–0.27	0.12	–0.22	0.21
ICAM-1	0.20	0.25	0.30	0.08
VCAM-1	0.17	0.35	0.21	0.22
ET-1	–0.16	0.38	–0.07	0.70
sP-selectin	–0.03	0.85	0.25	0.14
	Reference		**Target**	
	R (n=29)	***P***	**R (n=28)**	***P***
CD11b/CD18	0.15	0.43	–0.08	0.68

There is no association between change in inflammatory markers at 1 hour and change in CFR in both vessels in response to PCI as shown in Table 5.5.

Table 5.6 Relationship between change in inflammatory markers at 1 hour compared with pre-PCI levels expressed as inflammatory marker at 1 hour/inflammatory marker pre and change in IMR expressed as IMR post/IMR pre-PCI. Spearman's rank coefficient. *$P<0.05$

1 hour Post/pre	**IMR Reference Post/pre PCI** R (n =34)	*P*	**$IMR_{corrected}$ Target Post/pre PCI** R (n=35)	*P*
HsCRP	–0.16	0.36	–0.16	0.36
IL-6	–0.04	0.81	0.06	0.73
ICAM-1	–0.34	0.05	–0.31	0.07
VCAM-1	0.21	0.24	–0.17	0.33
ET-1	0.14	0.43	0.09	0.62
sP-selectin	–0.22	0.22	0.14	0.42
	IMR Reference Post/pre PCI R (n =29)	*P*	**$IMR_{corrected}$ Target Post/pre PCI** R (n=28)	*P*
CD11b/CD18	–0.11	0.56	–0.26	0.18

There is no relationship between change in inflammatory markers at 1 hour post-PCI and change in IMR as shown in Table 5.6.

5.4.5 Comparison between the ratio of inflammatory response at 24 hours vs. change in microvascular function post/pre PCI

Table 5.7 Relationship between change in inflammatory markers at 24 hour post-PCI expressed as inflammatory marker 24 hours post-PCI/pre-PCI levels and change in CFR expressed as CFRpost/CFRpre-PCI. Pearson's correlation coefficient.

1 hour Post/Pre	**CFR Reference Post/pre PCI R (n=34)**	***P***	**CFR Target Post/pre PCI R (n=35)**	***P***
HsCRP	–0.03	0.89	–0.04	0.84
IL-6	–0.27	0.13	–0.16	0.35
ICAM-1	0.02	0.90	0.16	0.36
VCAM-1	–0.01	0.97	0.02	0.89
ET-1	–0.13	0.49	–0.1	0.58
SP-selectin	0.17	0.34	0.22	0.20
	CFR Reference Post/pre PCI R (n=29)	***P***	**CFR Target Post/pre PCI R (n=28)**	***P***
CD11b	–0.03	0.88	–0.10	0.62

There is no relationship between modification of CFR and modification of inflammation following PCI as shown in Table 5.7.

Table 5.8 Relationship between change in inflammatory markers at 24 hour compared with pre-PCI levels expressed as inflammatory marker at 24 hours/inflammatory marker pre-PCI vs. degree of change in IMR expressed as IMR post/IMR pre-PCI. Spearman's rank coefficient. *P<0.05

24 hour post/pre PCI	**IMR Reference Post/pre PCI**		**$IMR_{corrected}$ Target Post/pre PCI**	
	R (n =34)	***P***	**R (n=35)**	***P***
HsCRP	–0.22	0.22	**0.42***	**0.01**
IL-6	0.19	0.29	0.18	0.32
ICAM-1	–0.17	0.34	–0.09	0.61
VCAM-1	0.17	0.33	–0.01	0.96
ET-1	0.24	0.16	**0.34 ***	**0.04**
sP-selectin	–0.04	0.81	0.21	0.22
	IMR Reference Post/pre PCI		**$IMR_{corrected}$ Target Post/pre PCI**	
	R (n =29)	***P***	**R (n=28)**	***P***
CD11b/CD18	0.28	0.25	0.19	0.33

There is a positive association between both change in CRP and change in ET-1 at 24 hours and change in IMR, (R=0.42 P<0.05, R=0.34 P<0.05) respectively. There is no association between any of the other markers.

In summary we see no association between basal levels of inflammation and pre-PCI microvascular function as assessed by both CFR and IMR. There is also no association between basal inflammation and response to microvascular function following PCI in the target vessel. We do however see association between the degree of change in CRP at 24 hours and change in IMR. We also see similar association between change in ET-1 and change in IMR.

5.4.6 Comparison between of inflammation and resting microvascular resistance

While there is a correlation between the magnitude of inflammatory change and minimal microvascular resistance, given there is evidence that inflammation can affect basal microvascular tone (Fichtlscherer *et al.*, 2004). We assessed the relationship between inflammatory markers and resting IMR, which is a reflection of basal microvascular tone.

Table 5.9 Relationship between baseline inflammatory markers and pre-PCI resting IMR in the reference and target vessels. Spearmans rank coefficient.

Pre-PCI	**$IMR_{resting}$ Reference**		**$IMR_{resting\ corrected}$ Target**	
	R (n=37)	***P***	**R (n=35)**	***P***
HsCRP	0.25	0.16	–0.01	0.94
IL-6	–0.09	0.60	0.11	0.53
ICAM-1	–0.12	0.50	–0.02	0.91
VCAM-1	0.25	0.15	0.21	0.22
ET-1	0.23	0.20	0.23	0.19
sP-selectin	0.13	0.43	0.02	0.92
Baseline	**$IMR_{resting}$ Reference**		**$IMR_{resting\ corrected}$ Target**	
	R (n=30)	***P***	**R (n=28)**	***P***
CD11b/CD18	0.16	0.42	0.02	0.93

There is no relationship between baseline inflammatory markers and basal levels of microvascular resistance in either the target or reference vessel as shown in Table 5.9.

Table 5.10 Relationship between baseline inflammatory markers and change in resting IMR post-PCI in reference and target vessels. Spearman's rank coefficient. *$P<0.05$

Pre-PCI	$IMR_{resting}$ Reference Post/pre R (n=34) *P*		$IMR_{resting\ corrected}$ Post/pre Target R (n=35) *P*	
HsCRP	0.03	0.88	–0.12	0.48
IL-6	0.20	0.27	–0.07	0.69
ICAM-1	0.13	0.48	–0.04	0.84
VCAM-1	0.34	0.05	–0.11	0.55
ET-1	0.17	0.32	–0.24	0.18
sP-selectin	0.20	0.25	0.08	0.63
	$IMR_{resting}$ Post/pre Reference R (n=29) *P*		$IMR_{resting\ corrected}$ Post/pre Target R (n=28) *P*	
CD11b/CD18	–0.13	0.52	0.016	0.95

There is no relationship between baseline inflammatory markers and degree of change in microvascular function in both vessels.

Table 5.11 Relationship between change in inflammatory markers at 24 hours and change in resting IMR in reference and target vessels following PCI. Spearman's rank coefficient. *P<0.05

24 hours Post/pre	$IMR_{resting}$ Post/pre Reference R (n=34)	P	$IMR_{resting\ corrected}$ Post/pre Target R (n=35)	P
HsCRP	–0.20	0.24	0.14	0.44
IL-6	–0.22	0.21	0.13	0.46
ICAM-1	–0.06	0.73	–0.17	0.33
VCAM-1	0.07	0.69	0.04	0.80
ET-1	0.11	0.54	0.07	0.67
sP-selectin	0.15	0.40	**0.39***	**0.02**
	$IMR_{resting}$ Post/pre reference R (n=29)	P	$IMR_{resting\ corrected}$ Post/pre Target R (n=28)	P
CD11b/CD18	0.19	0.33	0.03	0.90

There is a positive association between the activation of sP-selectin and change in IMR resting in the target vessel (R=0.39 and P<0.05) as shown in Table 5.11.

5.4.6 Periprocedural myocardial injury

11 out of 39 patients developed periprocedural myocardial injury,classified as troponin-T>0.04μg/l. All the patients in the study had troponin-T<0.01μg/l pre-PCI.

We analysed the relationship between baseline inflammatory markers and procedural factors to identify factors associated with peri-procedural myocardial injury.

Table 5.12 Relationship between inflammatory markers and procedural factors vs. troponin-T post-PCI as dependent variable. Spearman's rank $*P<0.05$ $**P<0.01$ $***P<0.001$

	R	n
CRP pre	–0.14	39
IL-6 pre	–0.04	39
ICAM-1 pre	0.017	39
VCAM-1 pre	**0.43****	**39**
ET-1 pre	–0.25	39
sP-selectin pre	–0.11	39
Neutrophil CD11b/CD18 pre	–0.07	34
CRP 24/pre	0.22	39
IL-6 24/pre	0.14	39
ICAM-1 24/pre	–0.08	39
VCAM-1 24/pre	0.03	39
ET-1 24/pre	**0.36***	**39**
sP-selectin 24/pre	–0.01	39
LDL pre	0.23	39
Inflation time	**0.50*****	**39**

FFR pre	–0.09	35
$IMR_{corrected}$ target pre	–0.28	35
$IMR_{corrected}$ target post	0.014	35
$IMR_{corrected}$ pre/post ratio	0.33	35

There is a relationship between VCAM-1 levels pre-PCI elevation of troponin-T post-PCI (R=0.43 $P<0.01$), and between degree of change of ET-1 and elevation of troponin-T. There is also a relationship between the total inflation time of the balloon during the angioplasty and troponin-T post-PCI (R=0.5 $P<0.001$).

5.4.7 Linear regression model to assess the relationship between change in IMR and independent variables

Since we are interested in factors which may account for microvascular disturbance following PCI, we performed multivariate linear regression analysis to determine which factors are associated with deterioration in microvascular function. We naturally log transformed non-normally distributed data and performed univariate linear regression analysis to determine which variables to include in the multivariate linear regression model. This model was then used determine which combination of explanatory variables related to the outcome variable IMR. Change in IMR pre/post-

PCI is the dependent variable. If $P<0.25$ in the univariate analysis then these variables were retained for inclusion in the multivariate model.

Table 5.13 Univariate regression between log IMR (dependent) post/pre ratio vs. independent variables in table. $*P<0.05$ $**P<0.01$ $***P<0.001$

Age	R= –0.19	*P*= 0.28	n=35
BMI	R= –0.07	*P*=0.69	n=35
LDL	R= 0.35	*P*=0.06	n=35
CRP pre	R= –0.26	*P*=0.13	n=35
IL-6 pre	R= –0.16	*P*=0.36	n=35
ICAM-1 pre	R= 0.15	*P*=0.38	n=35
VCAM-1 pre	R=0.09	*P*=0.62	n=35
ET-1 pre	R= –0.22	*P*=0.21	n=35
sP-selectin pre	R= –0.04	*P*=0.81	n=35
CD11b/CD18 neutrophil pre	R=0.21	*P*=0.28	n=28
Duration of balloon inflation	R=0.25	*P*=0.15	n=35
FFR Target pre	R=0.06	*P*=0.75	n=35
CFR Target pre	R=0.15	*P*=0.40	n=35
IMR Reference log pre	R= –0.28	*P*=0.10	n=35
IMR Target Log pre	**R= –0.73*** **	***P*<0.001**	**n=35**

CFI	R= 0.33	*P*=0.05	n=35

In the univariate analysis we find that log IMR target vessel pre-PCI is the only variable pre-PCI which is associated with change in IMR as shown in Table 5.13.

Figure 5.8 Relationship between log $IMR_{corrected}$ target vessel pre-PCI vs. log change $IMR_{corrected}$ target vessel

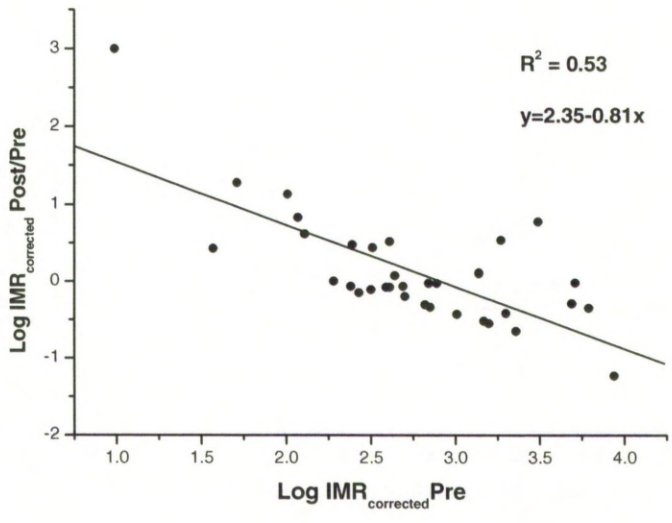


Figure 5.8 demonstrates association between log IMR target vessel pre-PCI vs. the degree of change in IMR post-PCI. Univariate linear regression analysis shows a close relationship between the two variables. R=0.73 R^2 =0.53 y=2.35–0.81x $P<0.001$.

We proceeded to input all the pre-PCI variables with a $p<0.25$ into a multivariate linear regression model. We put the following independent variables into the model; CFI, log IMR reference pre, log $IMR_{corrected}$ target pre, duration of balloon inflation, ET-1 pre, CRP pre and LDL. The dependent variable was log $IMR_{corrected}$ post/pre ratio. In the multivariate model, log $IMR_{corrected}$ pre was the only variable associated with log change $IMR_{corrected}$ post/pre ratio. $y=2.4-0.85x$ ($R^2 = 0.55$ $P<0.001$).

5.5 Discussion

Pre procedural increased inflammatory state in patients undergoing PCI has generated a lot of interest in view of data suggesting an increased risk of restenosis following PCI in subjects with higher levels of basal inflammation. There is a strong association between increased pre-procedural inflammation and death and recurrent ischaemia in patients undergoing PCI. This relationship is independent of the incidence of restenosis (Zairis *et al.*, 2002;Dibra *et al.*, 2003), hence suggesting an alternative mechanism to restenosis affecting the adverse outcome. In addition, the acute inflammatory response (i.e. the change in the level of circulating inflammatory markers) triggered by PCI is related to adverse outcomes after PCI (Buffon *et al.*, 1999;Walter *et al.*, 2001). Whilst there is an association between increased inflammatory response to PCI and the incidence of restenosis, this alone does not explain the increase in mortality and adverse events in this group of patients. The focus of interventional cardiologists has remained on epicardial vessel patency and there has been little research performed on the effect of inflammation on

microvascular function. This is the first study to investigate the association between inflammation and microvascular function in response to PCI with IMR.

We see a modest increase in CRP 24 hours post-PCI. There have been inconsistencies in previous studies, it seemed that in some patients there was increase in CRP post-PCI whereas in others there was little or no change, and the inflammatory response was seen in those whom had an already increased baseline levels of inflammation (Liuzzo *et al.*, 1998). Azar *et al.* demonstrated an increase in CRP in patients undergoing both PCI and coronary angiography but was more marked in PCI patients (Azar *et al.*, 1997). There were discordant findings from a small study which showed greater increase in CRP in patients with stable angina compared with unstable angina, which the authors suggested may be due to plaque disruption in stable patients, despite the unstable angina group having greater CRP to begin with (Almagor *et al.*, 2003).

Our patient group consisted solely of stable angina patients, and we saw a modest increase in CRP at 24 hours. Our rise in CRP is more in keeping with findings by Gautsenner-Wolf *et al.*, who demonstrated a 20-30% increase in CRP at 24 hours post-PCI (Gottsauner-Wolf *et al.*, 2000). A more marked inflammatory response is seen in patients with unstable angina (Tomoda and Aoki, 2001).

Our data show a marked early inflammatory rise in IL-6, which is similar to other studies. IL-6 begins to increase 15 minutes post inflammatory stimulus such as PCI,

and peaks at 2-3 hours (van Deventer *et al.*, 1990;Aggarwal *et al.*, 2003). In our study we do not see an overall change in ICAM-1 following PCI, and there is a very small increase in VCAM-1 post-PCI. Our data are similar to findings in a number of studies which failed to detect a rise in ICAM following PCI (Kurz *et al.*, 1994); (Ferns *et al.*, 2000;Mulvihill *et al.*, 2001). There is conflicting evidence on the effect of PCI on circulating ICAM-1. Some studies have suggested a rise in ICAM-1, particularly when sampled from the coronary sinus (Siminiak *et al.*, 1997), but there is conflicting data. Others have demonstrated no difference between coronary sinus and peripheral blood sampling when measuring ICAM-1 (Mulvihill *et al.*, 2001). The ARMYDA-CAM study did demonstrate an increase in ICAM-I levels post-PCI, this inflammatory response was attenuated by pre treatment with atorvastatin 40mg daily for one week prior to PCI (Patti *et al.*, 2006). It should be noted that the ARYMDA-CAM study did have patients with more complex lesions including total chronic occlusions and multi-vessel disease which may explain a more marked inflammatory response, since complex anatomy is often associated with more complex procedures and a greater degree of instrumentation, and longer segments of disease requiring stenting, all of which can increase the inflammatory response further. In our data we find inflation time is associated with periprocedural myocardial injury, supporting the notion increased instrumentation may cause increased secondary inflammatory response to myocardial necrosis.

We find no overall difference in circulating endothelin-1 following PCI. There are few data on the effect of PCI on circulating endothelin levels. Increased levels of endothelin-1 are associated with no-reflow in acute MI (Niccoli *et al.*, 2006). Blockade of endothelin, with both selective and non selective antagonists improves microvascular function in diabetics following PCI (Papadogeorgos *et al.*, 2009). In our study the lack of a rise in ET-1 post-PCI may not be surprising since endothelin-1 is preferentially secreted towards vascular smooth muscle cells rather than the lumen and seems to have more of a paracrine effect (Yoshimoto *et al.*, 1991).

There are conflicting reports of the effect of PCI on sP-selectin levels. Inoue *et al.* found an increase in sP-selectin following PCI in samples from the coronary sinus (Inoue *et al.*, 1999). There are a number of later studies suggesting no difference between coronary sinus sampling and peripheral blood sampling (Mulvihill *et al.*, 2001;Jaumdally *et al.*, 2007). Although the same investigators also found conflicting results on the effect of PCI on sP-selectin levels; Jaumdally *et al.* found an increase post-PCI whereas Mulvihill *et al.* found an acute reduction followed by an increase back to baseline levels at 24 hours. More recently an acute reduction in sP-selectin following PCI, which lasts for up to a week was seen in patients with stable angina (Munk *et al.*, 2011). In our study, we initially see an acute reduction in sP-selectin followed by an increase at 24 hours, back to pre-PCI levels. The ARMYDA 2 substudy attributed high dose (600mg) clopidogrel to reduction in sP-selectin post-PCI. This could explain our findings since all our patients were given 600mg

clopidogrel loading pre procedure instead of 300mg. In addition the administration of adenosine and heparin may have a significant effect upon sP-selectin. Unfractionated heparin increases activity of sP-selectin (Harding *et al.*, 2006). However, we see a reduction in sP-selectin immediately post-PCI. The use of adenosine may be an additional reason that we see an acute reduction of sP-selectin at one hour. Endogenous adenosine is known to reduce expression of P-selectin (Kitakaze *et al.*, 1991). Clearly there remains conflicting data on the effect of PCI on sP-selectin levels. It may be that circulating sP-selectin attaches to the activated endothelium in the myocardium, thus reducing the detectable amount in peripheral blood. The use of high dose clopidogrel and adenosine are important factors in our study.

We find an acute downregulation of the neutrophil MAC-1 receptor CD11b/CD18 expression 1 hour post-PCI, with a subsequent rise at 24 hours. Initial data on activation of neutrophil MAC-1 receptor shows PCI increases CD11b/CD18 expression, as demonstrated by early studies in which expression of CD11b/CD18 increased 15 minutes following coronary angioplasty (Inoue *et al.*, 1996;Serrano *et al.*, 1997). It is interesting that we see a down regulation in CD11b/CD18 post-PCI, our study only the second study recently to demonstrate these findings. More recently there has been evidence of early deactivation and down regulation CD11b/CD18 (Tiong *et al.*, 8 A.D.), which is similar to our data. The authors suggested modern adjunctive pharmacotherapy may be responsible for the attenuated inflammatory response. These unusual data may be due to the fact that most of the earlier studies

were performed before the current drug therapy regimes for PCI as discussed above. Also there has been a significant improvement in stent technology. We may be detecting the combined benefits of these advances related to PCI in these patients. However, a down regulation would suggest that CD11b/CD18 is deactivated. It is much easier to understand a lack of increase in CD11b/CD18 but a deactivation would suggest that some of the interventions currently used have the reverse effect.

In summary the dampened inflammatory response may be due to a number of factors, firstly all of our patients had stable angina and therefore a degree of plaque stability and less disruption of plaque during the procedure as well as less pro-inflammatory factors released from the plaque itself during the procedure. Secondly we have not controlled for all the baseline characteristics which may have an effect on the inflammatory response. Thirdly in the contemporary age of PCI, many additional drugs are used which are now considered routine for the procedure, such as the loading of dual anti-platelet therapy, and drugs used for primary or secondary prevention, such as high dose statins and ACE-inhibitors. Statins attenuate inflammatory response and reduce adverse events such as myocardial infarction and procedure related complications in patients undergoing revascularisation (Briguori *et al.*, 2004;Pasceri *et al.*, 2004;Radaelli *et al.*, 2007). The JUPITER study recently demonstrated the addition of a statin to patients with elevated CRP but normal LDL reduced cardiovascular events (Ridker *et al.*, 2008). There is now evidence that clopidogrel also attenuates inflammatory response to PCI (Vivekananthan *et al.*, 2004). In our

study all the patients were pre-treated with dual anti-platelet therapy as is now the norm. High dose clopidogrel has been shown to further attenuate inflammatory response (Klinkhardt *et al.*, 2002;Patti *et al.*, 2011). In addition 94.9% of patients were established on a statin and 46% were taking an ACE-I and an additional 10% were on angiotensin II receptor blocker. The high percentage of patients treated with stains and ACE-I in our group may account for the attenuated response in inflammation following PCI compared with other studies.

We fail to see an association between pre-PCI IMR and basal inflammation in our study. This is in contrast to a small study by Tomai *et al.*, where they found increased basal CRP is associated with impaired endothelial and non-endothelial-dependent microvascular function (Tomai *et al.*, 2005). Date *et al.* also found an association between IL-6 produced in the myocardium and increased microvascular resistance with the Doppler wire in patients with normal coronary arteries (Date *et al.*, 2005). Conversely no relationship was found between circulating inflammatory markers and CFR in women with chest pain and no obstructive CAD (Marroquin *et al.*, 2005). More recently patients with chronic active inflammatory disease such as rheumatoid arthritis and lupus were found to have impaired microvascular function without the presence of significant epicardial disease, and the duration of disease was associated with lower CFR (Recio-Mayoral *et al.*, 2009). There are also some data suggesting that slow coronary flow is more prevalent in patients with increased inflammation (Li *et al.*, 2007), but there is no consensus as to the effect of inflammation on basal

microvascular function. Earlier work by many investigators suggested CRP directly causes endothelial dysfunction but it must be noted that a lot of the earlier in vitro studies where CRP was added to endothelial cells were flawed due to the presence of azides and lipopolysacharrides in the CRP samples, which themselves rather than CRP itself, were toxic to the endothelium. Although the effect of inflammation on basal microvascular function is disputed it should be noted that in our study all of our patients were pre treated with glyceryl dinitrate, which is essential for both FFR and IMR measurements. GTN acts as NO donor and this may have counteracted some of the effect of inflammation seen particularly on the microvascular endothelial function. When we measured basal microvascular function in the form of resting IMR there was still no association with inflammation.

Of greater interest is the predictive effect of basal inflammation on outcome of microvascular resistance. Baseline CRP has predicted poor outcome in PCI in a number of different clinical settings (Buffon *et al.*, 1999;Saadeddin *et al.*, 2002;Palmerini *et al.*, 2005). In a pooled analysis of the four ISAR trials including over 4847 patients, high CRP predicted one year mortality and MACE *(Iijima et al., 2009).*

In our study we do not see any association between baseline levels of inflammation and outcome in degree of change in IMR. This is the first study of its kind specifically looking at outcome of IMR in association with inflammation, although there are a lot

of data suggesting baseline inflammation does affect outcome of PCI adversely. Buffon *et al.* demonstrated an increase in immediate post procedural complications and late restenosis in patients with elevated CRP pre procedure. Versaci *et al.* found a large increase in mortality and MI at 12 months follow up in the high CRP group of unstable angina patients undergoing PCI (Versaci *et al.*, 2000). Likewise Chew at al. found a 3 fold increase in death or MI at 12 months in patients with high CRP compared with normal CRP (Chew *et al.*, 2001b). Another large study of 500 patients with stable angina showed over 3-fold increase in combined endpoint of death, MI, urgent revascularisation and admission to hospital for unstable angina in those with elevated CRP at 2 year follow up. Interestingly they found no association with restenosis in high CRP group (de Winter *et al.*, 2002). Concordant results were seen in a study which showed a striking difference in mortality between high CRP and normal CRP, in patients undergoing unprotected left main coronary artery PCI at follow up of 9 months (Palmerini *et al.*, 2005). There are some studies that show basal CRP levels are not associated with poor outcome (Gach *et al.*, 2009). Similarly Saleh *et al.* could not find any adverse effect of elevated baseline CRP in 850 patients undergoing PCI, yet rather than baseline levels predicting poor outcome, they found the inflammatory response was predictive of adverse events including restenosis and new lesion development (Saleh and Tornvall, 2007).

Our data suggest that baseline levels of inflammation are not predictive of poor outcome of IMR in the target vessel in response to PCI, and this is true for all the

inflammatory markers, adhesion molecules and endothelin-1. The lack of association between baseline inflammation and outcome of IMR suggests that one of our main hypotheses is not supported. Baseline levels of inflammation do not predict outcome of microvascular function in patients undergoing PCI for stable angina. Although other studies as mentioned earlier did find strong evidence of increased CRP predictive of adverse events, the majority of these adverse events occurred after the first 24 hours, with the exception of Buffon's study, where the early events occurred during the procedure, or before discharge from hospital. In our study we are looking at immediate changes in the microcirculation post-PCI and assessing acute microvascular dysfunction. In addition we are looking at the specific outcome of change in IMR whereas there were many different medium to long term clinical endpoints such MI, death and revascularisation in the other studies. It seems increased basal levels of inflammation are less important than the inflammatory response to PCI.

In our study there is an association between the inflammatory response to PCI as assessed by circulating CRP and the modification in IMR (R=0.42 $P<0.05$). There is also a relationship between change in ET-1 and change in IMR in the target vessel (R=0.34 $P<0.05$). We do not see any other correlations with any of the other markers that were studied. Our results are supported by a number of studies which also show more of an adverse prognostic effect of the degree of inflammatory response to PCI rather than pre intervention levels, however none of these studies assessed microvascular function. There are no data on comparing inflammatory response with

change in IMR and our study is the first to report this. Although we failed to detect any relationship between basal inflammation and outcome of IMR, the relationship between inflammatory response and change in IMR is interesting. As suggested by experimental work by Griselli *et al.*, the inflammatory response rather than basal inflammation is much more challenging to myocardial salvage. Gach *et al.* showed in 89 patients, over 6 year follow up that a significant increase in CRP at the time of PCI is a stronger predictor for long term mortality than pre-PCI levels (Gach *et al.*, 2007). Two other studies have shown that the persistent elevated inflammatory markers 72-96 hours post-PCI are poor prognostic indicators. Patients with sustained elevation of detectable inflammation have a 20% incidence of combined death, MI and recurrence of symptoms vs. none, in patients in whom CRP has returned to normal at 72 hrs (Gaspardone *et al.*, 1998). Persistent elevated CRP at 96 hours is also a predictor of angiographic restenosis at 6 months (Gottsauner-Wolf *et al.*, 2000). Saleh *et al.* also found the degree of inflammatory response is an predictor of death or MI independent of myocardial necrosis in 891 patients (Saleh *et al.*, 2005).

The underlying mechanism behind the relationship between modification of CRP and modification of IMR is still unclear, since it cannot be said MR increases because of the acute inflammatory response, although that may be the case. However it could also be that microvascular damage causes a secondary elevation in CRP and hence may explain this finding. However there is no relationship between increase in IMR and periprocedural rise in troponin-T, which would suggest if anything an alternative

mechanism to myocardial necrosis may be responsible for the rise in inflammatory markers and rise in IMR. Also serum troponin-T level post procedure are not related to change in CRP in our study (r=0.21 P=0.19). Given that troponin-T levels are not associated with the increase in MR, this would also suggest that a mechanism independent of myocardial necrosis may responsible.

It is interesting that we also a relationship with endothelin-1 and IMR. It is possible that PCI releases vasoactive substances, such as endothelin-1, which are responsible for the increase in MR. However it should be noted that circulating endothelin-1 may not reflect endothelial and smooth muscle activity. Increased levels of endothelin-1 have been shown to be an independent predictor of the no-reflow phenomenon (Niccoli *et al.*, 2006). In addition, endothelin-1 blockade improves CFR post-PCI (Papadogeorgos *et al.*, 2009). The effect of endothelin on coronary microvascular dysfunction is an interesting area for further research.

The multiple regression model shows that the only pre-procedural factor associated to outcome of IMR in the target vessel is $IMR_{corrected}$ target pre-PCI. The lower IMR pre-PCI, the greater the degree of increase in IMR post-PCI. This is a strong relationship and does give rise to further questions. It is credible that the inflammatory response is associated with microvascular dysfunction for the reasons already discussed above, but the finding that very low IMR pre-PCI is a risk factor for worsening IMR is unexpected. It may be that those with very low IMR to start off with are those most at

risk, since the IMR is at the most minimal due to significant epicardial disease. Upon performing angioplasty, the large increase in pressure and flow may lead to a protective vasoconstriction, possibly via a myogenic response, thereby increasing MR post procedure.

Our analysis of inflammatory markers and CFR shows no correlations between baseline inflammatory markers and baseline CFR, and change in CFR post-PCI against baseline inflammatory markers, which is similar to our IMR data. However we also fail to see a relationship between change in inflammatory markers and change in CFR, as is seen with change in both CRP and ET-1, with change in IMR. This would be expected since IMR is a much superior method for measuring MR as discussed earlier. When we compare change in sP-selectin vs. change in IMR resting in the target vessel we find a positive association ($R=0.39$ $P<0.05$). This may not be surprising since sP-selectin is a key mediator in leukocyte rolling on the endothelium in response to endothelial activation. It should however be noted that resting IMR has similar methodological flaws to CFR, since basal microvascular tone and basal coronary flow are variable.

We have found relationships between baseline VCAM-1 and duration of balloon occlusion correlating with increase in troponin-T post-PCI. This may support the notion that increased activation of VCAM-1 will allow for greater myocardial necrosis following PCI due to increased adhesiveness of the endothelium distally. The

association between length of time balloon occlusion may be confounded by the fact that more complex procedures generally require a greater number of inflations and hence were more likely to give rise periprocedural myocardial necrosis. These findings are concordant with those of a number of studies which have all demonstrated that increased number of inflations increases the risk of periprocedural MI (Iakovou *et al.*, 2003). This could be because of more ischaemia with increased number inflations, yet a few seconds of ischaemia are on their own insufficient to cause myocardial necrosis. Our original premise of the cessation of flow being central to creating the ideal scenario for inflammatory mechanisms to cause microvascular dysfunction, could explain why we see this phenomena with increased number of inflations.

Conclusion

This is first study to describe a relationship between IMR and inflammatory markers and the relationship to the inflammatory response to PCI. There is a relationship between modification of CRP and ET-1 and modification of IMR only seen in target vessel, suggesting that there may be a locally acting phenomenon responsible in relation to PCI. However whether this is due to the inflammatory response having an adverse effect on the microcirculation, or whether the inflammatory response is secondary to another mechanism, and the subsequent microvascular injury causes a secondary inflammatory response is unclear.

There is no relationship between baseline inflammatory markers and change in IMR, suggesting that baseline inflammatory cannot be used to predict outcome of IMR post-PCI, this also suggests main hypothesis is not supported.

Low IMR pre procedure is predictive of worsening of IMR post-PCI, which is a novel finding, worthy of further investigation to establish the underlying mechanism.

5.6 Limitations

There are a number of limitations to our study. We used venous blood sampling instead of coronary sinus blood sampling, there is some evidence of more sensitivity in CS sampling of inflammatory markers (Inoue *et al.*, 1999). However, there are a number of studies which demonstrate little difference between CS and peripheral sampling (Mulvihill *et al.*, 2001;Jaumdally *et al.*, 2007). Hence we thought it was acceptable to use peripheral markers for estimates of local activation of inflammation as well as generalised inflammatory state.

We only sampled blood pre, 1 hour post and 24 hours post-PCI. We know that CRP peaks at 48 hours, and at 1 hour there is little change post inflammatory stimulus in most markers except IL-6, although changes are detectable within 24 hours in CRP, for ICAM, VCAM-1, sP-selectin and neutrophil CD11b/CD18.

The use of adenosine may attenuate inflammatory response, and this drug was used in all patients to achieve hyperaemia. If adenosine was responsible for affecting the inflammatory response, then we would expect to see a stronger relationship between modification of IMR and modification of CRP.

All our study patients underwent the procedure from the radial artery and the effect of radial artery cannulation for the purpose of coronary angioplasty may have contributed to the inflammatory response, however in our study group there were no cases of radial artery spasm requiring treatment with vasodilators. Since all the patients underwent the procedure from the radial artery route and there was no clinically significant radial artery spasm any effect on inflammatory response due to radial artery sheath insertion would be expected to be similar throughout the study population.

Chapter 6 Imaging myocardial inflammation

6.1 Introduction

According to our original notion, we would be able to predict an increase in inflammatory activity in the myocardial microcirculation of the territory supplied by the vessel undergoing coronary angioplasty in those patients with an increase in microvascular resistance following PCI. We have attempted to detect localised myocardial microvascular inflammation following angioplasty for evidence of active areas of suspected increased inflammation. Although there are many potential methods of imaging active inflammation, detecting inflammatory changes in the myocardium can be challenging owing to factors such as the availability of an agent which binds to inflamed tissue and can be readily detected by imaging techniques in humans. It is because of these limitations that *in-vivo* human data is limited. Despite these difficulties there are some useful methods available which are briefly reviewed.

Contrast-enhanced MRI has been utilised to detect myocardial inflammation especially in the diagnosis of acute viral myocarditis (Friedrich *et al.*, 1998b). Gadolinium-diethyl-enetriamine penta-acetic acid (Gd-DTPA) enhances inflamed tissue by penetrating to the extracellular space due to increased permeability of the vascular wall in inflammation (Roditi *et al.*, 2000). Gd-DTPA is detected as signal enhancement with MRI scanning and suggests the presence of inflammation. Similar

changes may be seen with acute myocardial ischaemia and infarction. It is possible to differentiate ischaemia from myocarditis by the distribution of late enhancement, which is predominantly subendocardial in ischaemia versus epicardial and patchy in myocarditis (Laissy *et al.*, 2005).

More recently there has been the use of contrast enhanced MRI using ultra-small superparamagnetic iron-oxide (USPIO) to detect inflammation in carotid plaque (Trivedi *et al.*, 2006). USPIO particles are taken up by plaque macrophages which can then be visualised within the plaque as signal intensity reduction. PET imaging with F18 fluorodeoxglucose (F18DG) has been used to demonstrate active inflammation in atherosclerotic plaque of arterial vessels. F18DG allows for imaging since inflammatory cells have much higher rate of glucose metabolism compared with neighbouring cell types. However it remains difficult to visualise inflammation in coronary arteries due to their small size and motion, and the fact that normal myocardium also takes up F18DG, hence making it difficult to differentiate changes related to the coronary vasculature (Rudd *et al.*, 2010).

PET/CT has been shown to identify vascular wall inflammation in larger blood vessels such as the common carotid artery and has been used to detect temporal arteritis. This technique utilises the ligand [^{11}C]-PK11195, which binds to the peripheral benzodiazepine receptor which is highly expressed in macrophages (Pugliese *et al.*, 2010).

The techniques described above demonstrate myocardial inflammation, or inflammation within atherosclerotic plaque and myocardial necrosis. However, we wish to detect inflammation in the microcirculation downstream from where the PCI was performed. We are interested in inflammation on the surface of the endothelium of the vasculature, the detection of which is more challenging.

It is possible to detect and quantify myocardial microvascular inflammation using microbubbles which can adhere to leukocytes in experimental models (Lindner *et al.*, 2000a). This technique was used in a canine model of ischaemia reperfusion whereby lipid microbubbles targeted towards leukocytes demonstrated areas of microvascular inflammation (Christiansen *et al.*, 2002). It is also possible to label echogenic microbubbles targeted at specific cellular adhesion molecule, for example ICAM-1 specific ligands have been used to detect transplant rejection in rats (Weller *et al.*, 2003). Other ligands to cellular adhesion molecules such a VCAM-1 can detect inflammatory changes on the aortic wall in experimental models (Kaufmann *et al.*, 2007). Most recently it has been possible to detect pre-atherosclerotic activation of VCAM-1 and sP-selectin using labelled microspheres and echocardiography (Kaufmann *et al.*, 2010). Despite exciting future prospects for detection of vascular inflammation, especially in the field of molecular imaging, there are sparse data in humans because there is a lack of contrast agents with ligands of proven safety and efficacy in humans.

More relevant to studies in humans than the above experimental approaches, is the well-established technique of imaging the distribution of radio labelled autologous leukocytes using gamma camera SPECT to detect localised inflammation. Neutrophils migrate to areas of acute inflammation, and ^{99m}Tc Hexamethylpropyleneamine Oxime (HMPAO) selectively binds to granulocytes in leukocyte population. The principle of blood cell labelling is that lipophilic radiometal-chelate complexes such as ^{99m}Tc-HMPAO are able to enter the cell membrane of the leukocytes. Once inside, the ^{99m}Tc-HMPAO is transformed into a hydrophilic complex and irreversibly bound (Peters, 1994a). This technique requires that leukocytes are separated from intravenous blood, and labelled in vitro. These labelled leukocytes can then be detected by gamma camera (Peters *et al.*, 1986). This technique has been used to detect occult infection and inflammation in clinical settings and has been particularly useful for patients with orthopaedic infections and inflammatory bowel disease. In addition, radiolabelled leukocytes have been used to detect myocardial abscesses around bioprothetic aortic valves (Salem *et al.*, 2004) and myocarditis (Yen and Yeh, 1993;Sun *et al.*, 2003). A similar technique was used with Indium-111 oxine to demonstrate myocardial inflammation in response to acute MI (Bell *et al.*, 1987).

To investigate the possible mechanisms of downstream arterial effects, we employed radio-labelling of leukocytes with subsequent visualisation by SPECT scanning with ^{99m}Tc-HMPAO-WBC due to superior quality of images and reduced radiation dose.

We propose post angioplasty inflammatory activation in the territory of the vessel which has undergone angioplasty in the form of activated endothelium and leukocyte complexes on the vessel walls of the microcirculation. It should be possible to detect this inflammation by imaging the bio-distribution of radiolabelled autologous leukocytes using a gamma camera. We chose to use this method as it is well established and safe for use in humans, and has the added advantage of being readily available locally.

6.2 Methods

We performed a pilot study on the first 9 patients investigated. Each patient underwent radiolabelled leukocyte scans on two occasions; the first 7 days prior to PCI and the second 24 hours post-PCI. SPECT scanning was performed 24 hours after reinjection of radiolabelled leukocytes. For detailed methods see Chapter 2 page 76.

6.3 Results

6.3.1 Visual Analysis

One patient refused to be scanned following reinjection of the ^{99m}Tc-HMPAO-WBC^{99m} due to claustrophobia. In all 17 datasets acquired there was no observed evidence of ^{99m}Tc-HMPAO WBC focal uptake in the region of the myocardium.as shown in Figure 6.1.

Figure 6.1 Example of visual analysis of uptake of ^{99m}Tc-HMPAO WBC

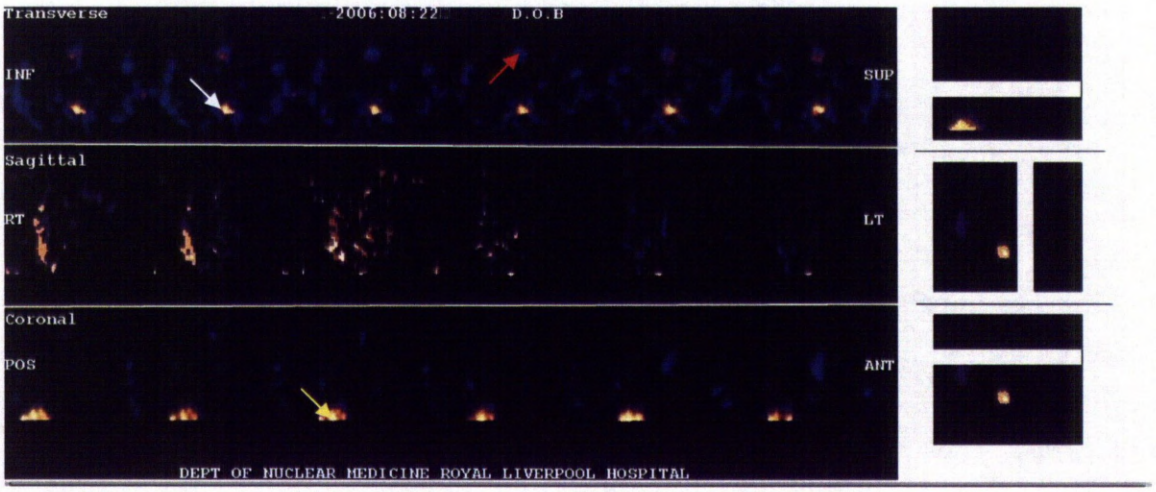


Figure 6.1 demonstrates the results of the SPECT scans. Bright signals are seen where uptake of leukocytes are detected, predominantly in the liver, spleen and bone marrow. Slices are chosen in the region of the myocardium. In the transverse slices the bright uptake seen in the spine (white arrow) and the sternum (red arrow). The slices seen at the bottom represent coronal slices, bright uptake is seen inferiorly in the liver (yellow arrow). There is no visual uptake seen in the region of the myocardium in all patients.

6.3.2 Quantitative analysis of myocardial inflammation

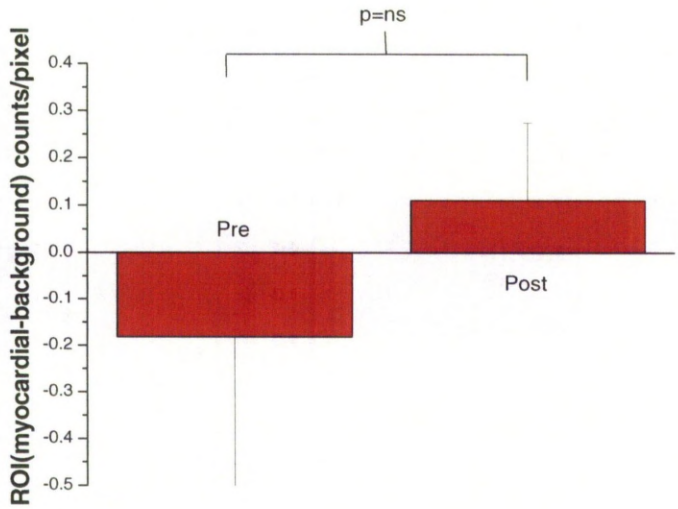
Table 6.1 Summary of quantitative detection of myocardial inflammation with ^{99m}Tc-HMPAO-WBC in 8 patients in response to PCI. Data is presented as Mean +/– SD

Patient	Radiation dose Activity (MBq)		ROI (myocardial-background) (counts/pixel)	
	Pre	Post	Pre	Post
1	204.0	169.8	–0.37	0.12
2	194.3	178.3	1.84	0.18
3	194.3	172.0	–0.02	1.19
4	165.2	137.8	–0.31	–0.01
5	161.9	160.8	–0.56	0.20
6	118.9	183.3	–0.17	0.18
7	185.0	194.3	0.41	0.18
8	183.4	192.6	–1.73	–0.50
Mean+/–SEM (95%CI)	175.9+/–9.6 (153.2-198.6)	173.6+/–6.5 (158.2-188.9)	–0.11+/–0.16 (–0.95-0.72)	0.18+/–0.35 (–0.2-0.5)

Table 6.1 shows that the mean counts/pixel of the summated slices is close to zero in all data sets. The maximum uptake seen in our patient goup is 1.8 counts/pixel, this is the equivalent of 0.002 counts/mm^3 and the mean counts/mm^3 for the difference between the Post PCI (myocardial ROI and background ROI) –Pre PCI(myocardial

ROI and background ROI) is 0.0003 counts/mm^3. This level of activity can be considered to be no more than normal background levels.

Figure 6.2 Effect of PCI on myocardial inflammation presented as mean counts/pixel (background-ROI). Mean+/–SD, n=8



There is no significant difference between the ROI(myocardial-background) before and after PCI. (0.19+/–0.16 counts/pixel vs. –0.11+/–0.35 counts/pixel n=8 *P*=0.38)

Discussion

This is a novel approach to identifying myocardial vascular inflammation, although there a few reports of similar techniques being used in the past to detect myocardial inflammation in response to myocardial infarction (Davies *et al.*, 1981;Bell *et al.*, 1987). This is the first study which attempts to image myocardial inflammation in response to coronary angioplasty. We did not detect any visual uptake of leukocytes in the myocardium using the technique described, and even with quantification of detectable uptake was negligible. Since counts/pixel were close to zero in all cases the values which were detected are can be considered to be no more than background levels. These negative results could be explained by the following possibilities;

There is no significant adherence of leukocytes on to endothelium (as determined by myocardial uptake of radiolabelled leukocytes), suggesting that the original hypothesis of activated endothelium allowing for this process to occur downstream does not occur in the setting of angioplasty. However, given the limitations of the technique used, failure to detect inflammation does not rule out the possibility of microvascular inflammation following PCI. There are other potential explanations for these lack of positive findings.

Leukocyte adherence is transient and the leukocytes were no longer adherent by the imaging time 24 hours after the acute event. However as previous studies have suggested, optimal timing for scans is 24 hours post insult although the timing of

injection of labelled cells should be as soon as feasible post insult (Davies *et al.*, 1981), this seems unlikely. Our patients were injected with the radilolabelled leukocytes 1hour post angioplasty and the images obtained 24 hours later to allow for clearing of labelled leukocytes from the blood pool, as these would have obscured any additional uptake by the myocardium.

Finally and perhaps most likely, the short duration of ischaemia in this study resulted in insufficient leukocyte adherence onto the endothelium to allow detection by the method used. The data previously presented that led to our use of this technique described models of ischaemia reperfusion whereby up to 60 minutes of ischaemia had been induced followed by reperfusion (Christiansen *et al.*, 2002). In our patients, the ischaemia induced lasts for no more than a few minutes at the most. Furthermore in the human studies, patients had imaging performed after established myocardial infarction secondary to prolonged ischaemia (Bell *et al.*, 1987) (Davies *et al.*, 1981).

Limitations

The technique used may not have the sensitivity required to detect inflammation at the microvascular level. However, it is a challenge to find the ideal imaging modality in humans, particularly if one is interested in microvascular inflammation. The use of a SPECT/CT acquisition rather than just a SPECT acquisition could have enabled anatomical mapping of the myocardial anatomy and permit localisation of any observed uptake in the myocardium and increase accuracy in the quantification. But

since there was no uptake seen on plain SPECT imaging, this would have been little additional benefit with higher radiation doses for the subjects. If SPECT/CT would have been available locally at the start of the study we could have utilised this. The number of patients in this study was small, 2 patients in this subgroup had evidence of periprocedural myocardial injury but there was still no visible uptake of radioisotope.

Chapter 7 Conclusions

Our aim was to describe the effect of PCI on microvascular function in patients undergoing coronary angioplasty for stable angina, and to identify mechanisms for microvascular disturbance with a view to potential clinical therapy. This study contributes to the understanding of pathophysiological mechanisms of microvascular injury following PCI, and for the first time demonstrates that PCI has an adverse effect on IMR in patients with stable angina. Furthermore we have found a complex interaction between the microcirculation in adjacent vessels during PCI. This study also compares the association between inflammation and microvascular function in relation to PCI. We showed pre-PCI inflammatory markers cannot be used as a predictor of outcome of microvascular function post-PCI, but there is an association between the degree of inflammatory response to PCI and the degree of microvascular dysfunction, when CRP and ET-1 are measured. Low pre-procedural microvascular resistance predicts patients with an increase in IMR and the only procedural factor which is associated with increase in IMR is the total duration of balloon inflation. One of the most significant results in this study is the reduction in IMR in the reference vessel following PCI in the adjacent vessel, which is a novel finding.

Microvascular dysfunction in PCI is of major interest and this study helps to further understand the underlying mechanisms. Our original hypothesis was

based on inflammation being a key mediator of microvascular dysfunction and we expected increased levels of baseline inflammation would help to identify those patients most at risk of microvascular dysfunction. From our data this notion is not supported, we do however see an association between inflammatory activation and microvascular dysfunction in response to PCI, when CRP and ET-1 are measured.

It is apparent that from our data that locally acting mechanisms are responsible for microvascular disturbance in response to PCI. We suggest that microvascular disturbance is occurring due to PCI causing either a mechanical obstruction, or distal microcirculatory vasoconstriction. We suggest microvascular dysfunction would be due to either due to a release of vasoactive or prothrombotic mediators from the plaque, or from the microcirculation in response to ischaemia in response to balloon/stent inflation against the vessel wall disrupting atherosclerotic plaque. An alternative mechanism causing distal microcirculatory vasoconstriction may also occur via the activation of alpha adrenergic receptors. It is interesting, however, that the phenomenon of microvascular dysfunction seems to be independent of myocardial necrosis in our study, suggesting an alternative mechanism to myocardial necrosis responsible for the increase in inflammation and microvascular dysfunction.

Release of endothelin from the plaque causing distal microcirculatory dysfunction is a possible mechanism supported by our data, alternative mechanisms include alpha adrenoreceptor mediated distal vasoconstriction in response to stretching of the epicardial vessel by PCI. We suggest a greater inflammatory response is seen in those with greater levels of plaque disruption, and hence any intervention which is able to stabilise the plaque during PCI may be beneficial. This may be the reason why statins are beneficial as pre-treatment before PCI.

The reference vessel reduction in microvascular resistance is an unexpected finding. We suggest this may be due to either an alpha adrenergic mediated microvascular vasodilation in the reference vessel territory or due to changes in collateral resistance. Another possibility is the release of other vasoactive mediators acting on the reference vessel bed.

We propose a unifying hypothesis which can explain these findings; Performing PCI causes microvascular dysfunction in a target vessel microvascular territory due to a release of vasoactive mediators from the plaque, the resultant increase in microavscular resistance in the target vessel causes a reduction of collateral resistance and thus a reduction in reference vessel resistance to compensate for the increase in target vessel resistance.

This study was designed to understand the mechanism behind microvascular function with a view to clinical benefit. Our data has got clinical relevance particularly in the way in which commonly performed intracoronary physiological measurements are interpreted in the form of FFR. FFR is an increasingly popular measurement used to guide the need for coronary intervention. FFR measurement assumes minimal microvascular resistance remains constant. We have shown in our study that minimal microvascular resistance can be variable and is affected by PCI, particularly where 2-vessel PCI is being considered. We now know that IMR can be influenced by PCI in adjacent vessel territories and hence interpretation of FFR in these settings must be done with some degree of caution, particularly if the FFR result is close to the threshold for intervention. Our study was not powered for clinical outcomes and it would be difficult to derive conclusions based on clinical outcomes given the small number of subjects. However any future studies should include follow up to detect major adverse cardiac events. It would be interesting to know how acute microvascular dysfunction at the time of PCI affects clinical outcomes and also to see how microvascular function responds in medium to short term if acute microvascular injury is sustained.

This study has raised a number of questions which would require further investigation and could have clinical benefit. Firstly the mechanism behind microvascular dysfunction in stable angina setting could be investigated by

assessing the role of endothelin and alpha adrenoreceptors. This could be done by the administration of the endothelin antagonist BQ123 and intra-corornary alpha-1 and alpha-2 adrenoreceptor antagonists respectively before PCI, and comparing this with control groups, and measuring changes in microvascular function. By sampling blood in the aorta, beyond the coronary lesion and in the microcirculation it could be assessed if vasoactive or prothrombotic mediators were released from the plaque or the microcirculation. These experiments would help to determine further the mechanisms responsible for peri-procedural microvascular dysfunction. The mechanism responsible for a reduction in IMR in the reference vessel can be tested by measuring IMR in all three major coronary arteries and assessing changes in collateral flow resistance and effects modifying alpha adrenergic activity and assessing response on IMR.

In summary the work presented here has 4 major findings, firstly there is an association between inflammatory activation when measured by CRP and ET-1 and microvascular resistance. Baseline levels of inflammation do not predict which patients will develop increase in microvascular resistance. The only pre procedural factor associated with an increase in IMR is low IMR pre-procedure. There is a reduction in IMR in the reference vessel in response to PCI in the target vessel. This work further questions the mechanisms underlying microvascular dysfunction in PCI and suggests the role of inflammation is more a response rather than a cause of the mechanism responsible for microvascular

dysfunction. Given that PCI in stable angina is predominantly a symptomatic treatment, any adverse effects from the procedure should be identified and minimised. This study adds to the understanding in this area and we have suggested areas for further development and research as outlined above.

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