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REMOTE ISCHAEMIC PRECONDITIONING IN THE CLINICAL SETTINGS OF CARDIAC BYPASS SURGERY AND CORONARY ANGIOPLASTY

by

Dr Girish Ganesha Babu

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Approved by _____

Chairperson of Supervisory Committee

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REMOTE ISCHAEMIC PRECONDITIONING IN THE CLINICAL SETTINGS OF CARDIAC BYPASS SURGERY AND CORONARY ANGIOPLASTY

By DR GIRISH GANESHA BABU

Chairperson of the Supervisory Committee:

Professor Derek M Yellon
The Hatter Cardiovascular Institute

A thesis presented on the cardio-protective properties of Remote Ischaemic Preconditioning in different settings of myocardial revascularisation, namely during coronary artery bypass surgery and during urgent coronary angioplasty.

DECLARATION

I, Girish Ganesha Babu, confirm that the work presented in this thesis is my own. Where information is derived from other sources, I confirm that this has been indicated in the thesis.

Signed

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1. INTRODUCTION

1.1 Coronary Heart Disease: Where Do We Stand

Over the past two decades, significant progress has been achieved in combating coronary heart disease (CHD), both in prevention and treatment. In the UK, death rates from CHD for people under 65 years old has fallen by 45% in the last decade(1). However, CHD continues to be the most common cause of premature death in the UK (1). CHD manifests in a spectrum of clinical presentations ranging from stable angina, acute myocardial infarction (AMI), heart failure (HF), arrhythmias and sudden cardiac death (SCD). The location, extent, and the nature of the CHD determine the clinical presentation in these patient groups. These factors also determine the definitive treatment the clinicians provide, which are tailored individually. Patients with stable angina can be treated pharmacologically to control symptoms. For more unstable presentations, myocardial revascularisation remains the main therapeutic option. Further, with the progression of CHD, many stable angina patients on medication will eventually require myocardial revascularisation. Therefore, myocardial revascularisation has occupied a prominent part in the treatment of cardiac patients. 'Myocardial revascularisation', which refers to the restoration of blood supply to the ischaemic myocardium, can be achieved by three main methods:

- Percutaneous Coronary Intervention (PCI)

- Coronary artery bypass grafting (CABG)

- Thrombolytic therapy (applicable for patients with acute myocardial infarctions, where primary percutaneous intervention facilities are not available).

Coronary atherosclerotic plaque status and its progression determines the clinical presentation and subsequent strategy of myocardial revascularisation. Atherosclerotic plaque obliterating > 50% of the lumen is considered significant stenosis and likely jeopardises the myocardial flow therefore necessitating one or the other form of revascularisation. These lesions are treatable with either PCI or CABG. A significant lesion in

more than two epicardial coronary arteries, or the involvement of Left main stem of the coronary circulation is dealt with CABG. Lesions involving up to two coronary arteries, and with favaourable lesion anatomy are dealt with PCI. In acute plaque rupture with platelet plugging and complete coronary acute obliteration (STEMI), an emergency PCI to open the artery is performed and is called primary PCI. Alternately, a strategy to pharmacologically thrombolyse the acute thrombosis is used in few geographical areas where primary PCI is unavailable.

Percutaneous Coronary Intervention (PCI) is an interventional procedure whereby the diseased narrow coronary arteries are opened percutaneously through the usage of catheters and balloons. In most cases, an intra-coronary stent is deployed at the site of the balloon angioplasty which maintains the lumen patency. PCI has become the predominant procedure for coronary revascularisation in patients with both stable and unstable CAD. Since the first description of coronary angioplasty by Andreas Grüntzig in 1977, the procedure has been modified extensively. The technical advances coupled with the use of coronary stents and adjuvant drug therapies have resulted in high procedural success rates and low re-stenosis rates. Older patients are now being treated and more complex multiple coronary lesions deemed appropriate for PCI.

Coronary artery bypass graft (CABG) surgery is the surgical method of coronary revascularisation, whereby vessels from another part of the body are harvested and grafted (proximal to and distal to the diseased coronary artery) to restore the blood supply to the ischaemic myocardium. CABG requires open thoracotomy and is done under general anesthesia. Over the last 2 decades a significant proportion of patients previously deemed appropriate for CABG are increasingly revascularised using PCI. However, CABG remains a predominant coronary revascularisation procedure in patients with more severe and complex coronary disease who are unsuitable for PCI.

1.2 Ischaemia Reperfusion Injury

1.2.1 Ischaemic Injury

Prolonged deprivation of blood supply to the myocardium results in ischaemic injury. Typical ischaemic insults in a clinical setting happen during an acute myocardial infarction, during percutaneous coronary interventions, and during cardiac surgery. The quantity of ischaemic damage sustained by the myocardial tissue depends on the duration of ischaemia, the area of myocardium at risk, and the presence of collateral blood supply to the affected myocardium.

At a cellular level, oxygen and substrate deprivation during ischaemia results in reduced oxidative phosphorylation and consequently reduced adenosine tri-phosphate (ATP) levels. Lack of ATP affects cell membrane ion channels leading to sodium (Na^+) and water accumulation and loss of potassium (K^+) from the cells, which result in progressive cellular swelling. The activation of anaerobic glycolytic pathways to compensate for the lack of energy generation through the respiratory chain results in intracellular acidosis due to lactate accumulation. This results in activation of sodium-hydrogen (Na^+H^+) exchanger resulting in further cellular Na^+ accumulation. In addition, the accumulation of fatty acid metabolites through lipolysis leads to progressive impairment of cell membrane structure and function(2). Sodium-Calcium exchanger (Na^+/Ca^{2+}) is one of the most important cellular mechanisms for removing Ca^{2+} and removes one calcium ion in exchange for the import of three Na+ ions. In response to high intracellular Na^+ it acts in reverse to maintain sodium homeostasis but results in increased intracellular Ca^{2+} accumulation. The Ca^{2+} entry into the cell also happens through direct Ca^{2+} channels and through the release of Ca^{2+} from cellular sarcoplasmic reticulum during ischaemic insult(3). The transition of reversible injury to irreversible injury begins with unregulated Ca^{2+} accumulation, which also is characterised by the development of severe defects in membrane permeability (4). The mitochondrial matrix Ca^{2+} levels also increase as the Ca^{2+} uniporter in the mitochondrial membrane acts as a channel through which extra-mitochondrial intracellular Ca^{2+} enters into the mitochondrial matrix. This leads to Ca^{2+} overload with loss of electrical potential difference across the membrane leading to mitochondrial damage (5). The classical necrotic cell death ensues with

swelling of cell organelles and consequential extrusion of all cellular contents into the extracellular matrix due to rupture of the plasma cell membranes.

In clinical settings, a purely ischaemic injury leading to necrosis of myocardial cell (infarction) can happen during an untreated myocardial infarction or a silent myocardial infarction. During elective coronary interventions in patients, peri-procedural micro-embolism of the atherosclerotic plaque debris is another instance where predominantly necrotic myocardial cell death happens. However, in most other clinical settings, the ischaemic myocardium is replenished with its blood supply by therapeutic or spontaneous revascularisation. This 'reperfusion', of course limits further ischaemic damage to the cell, however it also initiates a cascade of cellular events which lead to myocardial damage termed 'reperfusion injury'. This phenomenon will be discussed in more detail in section 1.2.

Apart from necrotic cell death, there are two other modes through which cell death can occur due to ischaemia reperfusion injury; 'apoptosis' and 'autophagy'. Apoptosis can be initiated by the release of cytochrome c from the damaged mitochondria (mitochondria initiated) or through ligation of a death receptor that transmit apoptotic signals within the cell. Both these apoptotic routes result in the activation of cytosol caspases. Cell death through this mechanism is mainly through cell shrinkage, condensation, margination of the chromatin, and budding of the plasma membrane (6). Ischaemia can also trigger a third method of cell death through autophagy, an intracellular phenomenon where the cell digests its own constituents. Knowledge of the exact mechanism of this type of cell death is still limited, but it involves proteolytic lysozymal enzyme activation in response to lactate and protons accumulation in the cell. Although the mechanisms of all three types of cell death are thought to be mutually exclusive, in practice considerable overlap exists between them, and individual dying cells may exhibit features of more than one mechanism over time (6).

1.2.2 Reperfusion Injury

Reperfusion of ischaemic myocardium contributes is necessary to prevent further ischaemic damage of the cells experiencing absolute myocardial ischaemia. However, on sudden reperfusion, the myocardial cells that have experienced varying degrees of ischaemic damage

initiate a cascade of cellular events, which can actually lead to further cellular damage and death. This phenomenon of myocardial cell injury and death, due to the sudden restoration of perfusion in an ischaemic myocardium is known as ''lethal reperfusion injury'.' Lethal reperfusion injury contributes to the final infarction size of the myocardium. Myocardial reperfusion can also lead to other clinical manifestations other than lethal reperfusion injury, namely myocardial stunning and reperfusion arrhythmias.

For lethal reperfusion injury to occur, ischaemia has to set the stage without producing irreversible injury itself. Therefore the corollary is that, ischaemic alterations of cellular conditions are necessary prerequisites for lethal reperfusion injury, but not in themselves sufficient causes for cell death (7). Unfortunately, in the setting of ischaemia-reperfusion, it is technically impossible to evaluate manifestation of irreversible injury in the same piece of myocardium both before and after reperfusion. It is also not possible to determine whether cell death is caused entirely by the ischaemic history or by reperfusion from analysis of developing damage in the reperfused myocardium alone (7). The only indirect way of establishing this is by studying whether modification of the conditions of reperfusion can prevent cell death that may otherwise occur in ischaemic-reperfused myocardium. It is now well established that some modifications to the reperfusion conditions do indeed provide protection.

The cellular mechanisms involved in reperfusion injury have been well elucidated over the years by many researchers and are reviewed in detail by Yellon and Hausenloy (8) and Piper *et al*. (7). Current proposed mechanisms of reperfusion injury involve: a) abrupt intracellular calcium overload leading to myocyte hyper-contracture b) oxidative stress c) rapid restoration of physiological pH from acidotic milieu and d) activation of inflammatory cascades, all of which would aid in reperfusion induced myocardial necrosis or apoptotic death.

Cardiac myocytes consume large quantities of energy. To accommodate this requirement, these cells host a high density of mitochondria. The mitochondrial permeability transition pore (mPTP) has been the centre of a growing amount of attention for its role in the mediation of reperfusion injury. The inner mitochondrial membrane, which is responsible for maintaining mitochondrial transmembrane potential, is normally impermeable to ions and proteins. Dissipation of the electrical potential across this membrane is termed

"permeability transition", a process thought to be mediated through the mPTP. Although the constituent protein components of the pore remain unknown, formation of the pore creates a nonselective channel between the inner membrane of the mitochondrion and the sarcoplasm. This results in loss of the electrochemical gradient, the release of reactive oxygen species (ROS), and apoptosome formation. Calcium overload, rapid normalization of pH and oxidative stress are all known to be triggers of mPTP. The non-specific highly permeable mPTP leads to uncoupling of oxidative phosphorylation and consequent ATP depletion, mitochondrial swelling and loss of cellular integrity (5, 9). Also, pro-apoptotic pathway activation by the release of cytochrome c from the inner mitochondrial membrane also contributes to cell death. With its central role in reperfusion injury, the inhibition of mPTP has emerged as a prime target in inhibiting myocardial reperfusion injury.

In clinical settings, it is not possible to sustain lethal reperfusion injury without ischaemic injury. Consequently most cases of myocardial reperfusion injury would also have varying degrees of myocardial ischaemic injury. Some classical ischaemia-reperfusion injury situations encountered in cardiac care include acute ST elevation myocardial infarction (STEMI) subjected to primary percutaneous intervention or thrombolysis and, in almost all cardiac surgeries, requiring cardioplegic arrest.

Over the years, the major priority in the management of acute MI has always been the early restoration of blood supply. This led to the rapid development of more advanced medical and interventional techniques for rapid restoration of reperfusion to limit the ischaemic myocardial injury. In cardiac surgery settings too, the emphasis has been in reducing the ischaemic time of the cardioplegic heart with improvement in both surgical techniques and instrumentation to limit ischaemic injury to the heart. However, a seminal paper by Murry *et al.* (10) provided scientists with an additional tool to further reduce infarct size by limiting myocardial ischaemia-reperfusion injury and heralded the era of ischaemic preconditioning.

1.3 Ischaemic Preconditioning

Although the pathogenesis of ischaemia-reperfusion injury was well understood in early years it was not until 1986 when canine experiments by Murry *et al.* (10) heralded the concept of "Ischaemic Preconditioning" - a phenomenon which is now understood to involve a wide range of cellular mechanisms and pathways. Using dog models, Murry *et al.* (10)

showed that by subjecting the hearts to four cycles of 5 minute regional ischaemia followed by 5 minute reperfusion and subjecting the same myocardium to 40 minute sustained ischaemia, the final infarct size was reduced by 75% when compared to control hearts.(10) This endogenous protective phenomenon, where brief episodes of sub-lethal ischaemia to an organ render it resistant to prolonged lethal ischaemic insult, is known as Ischaemic Preconditioning (IPC). Over the last 2 decades, significant progress has been achieved in elucidating the precise mechanism of this cardioprotective phenomenon and IPC has been shown to occur in virtually all animal species tested, including humans, and in most of the tissues including kidney (11), brain (12), lung (13), skeletal muscle (14), and liver (15).

The cardioprotection conferred by IPC of the heart reveals two distinct components – 'Classic preconditioning' and 'Delayed preconditioning'. The classic preconditioning phase is induced immediately after the sub-lethal ischaemic stimulus and lasts for 2 to 4 hours (16, 17). A second phase of 'delayed preconditioning' sets in about 24 hours after the initial preconditioning stimulus and lasts up to three days (18, 19). Delayed preconditioning is also called the 'second window of preconditioning (SWOP)' or 'late preconditioning'.

1.3.1 Mechanism of Ischaemic Preconditioning in Myocardium.

Although it has been widely believed that receptor mediation plays significant roles in preconditioning, there are sufficient uncertainties in the mechanism of IPC to conclude that IPC is largely receptor mediated. Receptor activation during IPC activates complex signaling cascades, which during the lethal ischaemia converge on one or more end-effectors to mediate the protection. Identification of the triggers, transducers and end effectors in the cardiac myocyte that are activated in IPC has been the major focus of research in the last two decades.

An important part in the preconditioning process is the cell's innate memory to the preconditioning stimulus, rendering the cell in a preconditioned state during this memory period. As discussed above, this memory period appears to be 2-4 hours in classic IPC and up to 72 hours after the delayed preconditioning has set in. The memory information in classic preconditioning is thought to occur as a reversible post-translational modification of some

pre-existing protein (such as a phosphorylation or translocation), but the site of that modification is unknown (20). In delayed preconditioning, an additional mechanism of new protein synthesis is also described. As described by Yellon *et al.* (20) in their review, the mechanism of preconditioning is probably best described in terms of triggers, mediators, memory, and end effectors. (20)

1.3.2 Mediators of 'Ischaemic Preconditioning' - Trigger Mechanism

The cell surface receptors, which, when stimulated by ischaemia act as trigger mechanism in the preconditioning process. Three main groups of cell surface receptors – G_i protein-coupled receptors (GPCR), growth factor receptors and other ligand specific receptors are thought to be involved in the preconditioning process. Although receptor mediated trigger largely accounts for IPC, there are also non-receptor mediated preconditioning triggers thought to be involved when the stimulus is non-ischaemic (i.e. volatile anaesthetics, Statins, metformin and mechanical stimuli like heat and stretch) (21). GPCRs on myocardial cell surfaces could be stimulated by their respective ligands, and many of the activated GPCRs can trigger a preconditioned state by activating tyrosine kinase. Adenosine A_1 GPCR was the first GPCR to be shown to play a role in ischaemic preconditioning (22), followed by bradykinin and opioid receptor ligands with a role in IPC (23, 24). Subsequently other GPCR ligands like urocortin, adrenomedullin and glucagon-like peptide-1 (GLP-1) were found to be associated with IPC. Growth factor receptor ligands associated with IPC include insulin, transforming growth factor (TGF)- β, insulin like growth factor (IGF)-1, corticotrophin 1, fibroblast growth factor (FGF)-2, granulocyte colony stimulating factor (G-CSF), erythropoietin and adipocytokines (visfatin, apelin, leptin) (21).

The third class of receptors involved in preconditioning are other ligand specific receptors like oestrogen receptor and guanyl cyclase receptor, which bind atrial natriuretic peptide(ANP) (21). These characterisations aid in targeting these receptors with pharmacological agents and, hence, mimic the effect of preconditioning.

1.3.3 Mediators of Ischaemic Preconditioning- Signal Transduction Pathways

At the sub-cellular level, many 'protein kinases' which get activated through the above mentioned trigger receptors act as mediators of signal transduction. While adenosine receptors directly activate protein kinase C (PKC), opioid and bradykinin receptors activate phosphoinositide 3-kinase (PI3K), which in turn activates serine/threonine kinase (Akt) (25). Subsequent downstream activation, in order include - activation of nitric oxide synthase (NOS) and nitric oxide (NO) formation, guanylate cyclase activation, protein kinase G (PKG) activation, and finally protein kinase C (PKC) activation (25). It is thought that activated PKC is the molecule that helps the cell 'remember' it has been preconditioned long after the trigger has ceased. Activation of PKC appears to need reactive oxygen species (ROS), which in turn is thought to be released through the opening of inner mitochondrial ATP-dependent potassium channels (mitoK$_{ATP}$). PKG appears to be the key mediator transmitting signals from the cytosol to the inner mitochondria; the exact mechanism is still not clear but appears to involve ε-PKC (26). The opening of mitoK$_{ATP}$ channels results in an influx of potassium that causes swelling of the mitochondria, which is thought to lead to the production of ROS, although the exact mechanism is still unclear (25). The production of ROS happens when there is reperfusion after a sub-lethal ischaemic stimulus. ROS acting as a second messenger at reperfusion in a PKC dependent mechanism are responsible for the generation of pro-survival kinases (Akt and ERK1/2). This Reversible Ischaemic Salvage Kinase (RISK) pathway confers cardioprotection at reperfusion by resisting the mitochondrial permeability pore (mPTP) opening (21). This confers antinecrotic, anti-apoptotic and anti-autophagic effects resulting in reduced cell death (27). It is important to realise that ROS production is not only associated with reperfusion injury after lethal ischaemic insult but also in the preconditioning cascade, which is actually cardioprotective.

1.3.4 Mediators of Ischaemic Preconditioning - End Effectors

The possible end-effector, on which many of the preconditioning pathways appear to converge, is the mitochondrial permeability transition pore (mPTP). As mPTP opening is the key phenomenon leading to cell death, inhibition of this pore opening by preconditioning

during reperfusion confers a large part of cardioprotection. The other end effectors that are thought to be involved in the preconditioning mechanism involve 'gap junctions' and 'sodium/hydrogen (Na^+/H^+) exchanger'. Preconditioning inhibits the Na^+/H^+ exchanger aiding cell resistance to osmotic swelling. Inhibition of the Na^+/H^+ exchanger would also prevent Na+/Ca2+ exchanger opening during reperfusion, hence, preventing Ca2+ influx related injury.

The gap junction plays a role not only in electrical coupling of cardiomyocytes but also in intercellular transport of biologically active substances. Furthermore, the gap junction participates in decision making on cell survival versus cell death in various types of cells, and may mediate part of reperfusion injury in the heart (28). Lethal ischaemia suppresses both electrical and chemical gap junction communication (GJC). IPC delays electrical GJC suppression by ischaemia, conferring cardioprotective effect against ischaemia reperfusion injury (29).

1.4 Ischaemic Postconditioning

In clinical practice, IPC poses a major limitation in its applicability, as it is not possible to predict the onset of index ischaemia. This applies to most clinical situations encountered involving ischaemia reperfusion injury, like acute myocardial infarction (AMI). However, in situations where the index ischaemia can be predicted, as in cardiac surgery, IPC potentially could be evaluated. IPC, by definition has to be induced before the lethal ischaemic insult.

Zhao *et al.* (30) in 2003, described a new phenomenon in their canine model, where rapid interruption of blood flow in the early phase of reperfusion after lethal ischaemia conferred a similar degree of protection as provided by IPC. This phenomenon of cardioprotection induced by interrupted perfusion of the already ischaemic tissue before continuous reperfusion is called Ischaemic post conditioning (IPostC).

In fact, Hausenloy *et al.* (31) in 2004, had demonstrated that IPC actually exerts its protection early in reperfusion following the lethal ischaemia, and gave a physiological basis as to why IPostC should work. The therapeutic time frame for IPostC is very discrete, within initial minutes of reperfusion. In animal models, a delay in postconditioning of more than one minute into reperfusion has failed to show cardioprotection (32). The mechanisms through which IPostC confers cardioprotection appear to be through similar mechanisms seen in IPC.

There are studies implicating the RISK pathway (Erk1/2 and PI3 Akt) in postconditioning. Similarly, many studies have shown the involvement of ROS, PKC, nitric oxide, mito-K_{ATP} and inhibition of mPTP (33). Certainly, the involvement of similar mediators and molecules in both IPC and IPostC indicate that they evidently have very different roles by virtue of the timing and the compartment in which their effects are exerted.

Cardioprotection from IPostC is, therefore, applicable in human settings as it can be applied after the onset of index ischaemia. Indeed, this new concept was soon applied in a clinical setting in a pilot study by Laskey *et al* (34). They showed improved ST segment shift response and Doppler-derived distal coronary velocity data in acute MI patients subjected to IPostC undergoing primary PCI. Later, Staat *et al* (35)*,* in similar classes of patients showed significant reduction in cardiac enzyme (total CK) release in patients subjected to IPostC. IPostC was induced through brief inflations of angioplasty balloon in the affected artery after reperfusion. The IPostC stimulus in this study was provided within 1 minute of coronary reflow and included inflation of angioplasty balloon for 1 minute followed by 1 minute reperfusion for 4 cycles.

The potential limitations in applicability of IPostC in routine practice in patients with acute MI surrounds the fact that it can only be applied to patients undergoing primary PCI and not thrombolysis. In addition, when thromboaspiration catheters are used in primary PCI, the utility of IpostC, and possible interference with IpostC, is uncertain (36). Certainly, pharmacological post-conditioning triggers would be much more applicable in all these settings, and consequently the search of pharmacological postconditioning agents continues. In this context, Ovize *et al.* (37) in their pilot study involving 58 patients showed that the drug Cyclosporin-A, used as a pharmacological postconditioning substance, reduces the final myocardial infarct size in patients undergoing primary PCI .

1.5 Remote Ischaemic Preconditioning

Seven years after the discovery of phenomenon of Ischaemic Preconditioning by Murry *et al.* (38), an intriguing discovery was made by Przyklenk *et al.* (39) in 1993 of a phenomenon in their canine model. They showed that prior induction of brief episodes of sub-lethal

ischaemia followed by reperfusion in circumflex artery territory, protected the myocardium supplied by left anterior descending artery (LAD) through reduction in the infarct size when LAD was occluded and reperfused (40). The initial explanation for this phenomenon was that a diffusion factor, which generates in the preconditioned tissue, would diffuse into remote tissue and provide protection. In addition, the possibility of stretch induced cardioprotective signaling was put forward as another explanation, but later studies have failed to identify such a local diffusion factor. The intra-cardiac protection of was later extended to other organs. A number of studies have identified the cardioprotective effect from preconditioning distant organs after an ischaemic insult, and also protection of other organs from ischaemic insult. This intriguing phenomenon, of distant organ protection from lethal ischaemic insult when a different remote organ is preconditioned prior the onset of lethal ischaemia is called Remote Ischaemic Preconditioning (RIPC)

Inter Organ Remote Preconditioning

McClanahan *et al.* (41) in their *in-vivo* rabbit model, were the first to demonstrate that a 10 minute left renal artery occlusion was as effective as a 5 minute brief coronary artery occlusion (classic IPC) in reducing infarct size. A study by Gho *et al.* (42) on rat models in 1996, was pivotal in further understanding of the remote preconditioning mechanism. In their study, they assessed the cardioprotective effect by inducing transient sub-lethal ischaemic stimulus to the small intestine and kidneys. They also showed that transient mesenteric artery occlusion of 15 minutes followed by 10 minutes reperfusion, before 60 minutes of coronary artery occlusion and 180 minutes of reperfusion, provides a similar reduction in infarct size as 15 minutes of classic myocardial preconditioning (IPC). In their renal model, the protection was conferred only in presence of hypothermia, but this was not necessary in mesenteric artery conferred protection. Further, they showed that the cardioprotective effect of mesenteric artery preconditioning is lost by administering hexamethonium - a ganglion blocker. In addition, they showed that cardioprotection is lost if the mesenteric artery occlusion was not followed by reperfusion. The results of this study provided the first mechanical insights into this intriguing phenomenon. An involvement of a humoral factor was suggested due to the fact that reperfusion was mandatory to confer distant protection. Meanwhile the attenuation of the cardioprotection by hexamethonium

also suggested the involvement of neural pathways in this phenomenon. Subsequent to these studies, more animal studies have shown cardioprotection by brief sub-lethal ischaemic exposure of renal, mesenteric, hind limb and carotid arteries (43). These studies are reviewed in detail by Hausenloy *et al*. Many of these studies have also contributed mechanistic insights into the RIPC phenomenon.

1.5.1 Mechanisms Underlying RIPC

Although several mechanisms are proposed to be involved in mechanistic pathways and signal transduction cascades, the exact nature of this remote organ protection phenomenon remains largely unclear. What is known so far, is that the cardioprotection mechanisms in the heart from RIPC have a lot similarity to the cardioprotection mechanisms through classic ischaemic preconditioning. Nevertheless, what heralds these protective signaling cascades at the outset in a remote organ and how this entity reaches the remote organ are still under research. The mechanisms known may be subdivided into: a) remote organ (preconditioned organ) responses to RIPC stimulus, b) cardioprotective message relay mechanisms conveyed from remote organ to heart, c) myocardial protective response to the relayed message from the remote organ.

Remote organ ischaemic responses involve release of endogenous autocoids and factors, which are responsible somehow for triggering cardioprotection involving signal relay mechanisms (44). Since the discovery of RIPC, the emphasis has been on the study of the role of relay mechanisms between the remote organ and the heart. Two major relay mechanisms have been postulated with evidence supporting both mechanisms. The first mechanism postulated, is the presence of a humoral factor released in the remote tissue, which is then transported to the heart. The second mechanism postulated, is the presence of a neural pathway between the remote organ and the myocardium, which acts as a signal relay medium.

1.5.1.1 Humoral Mechanism

The possibility of a potential humoral factor responsible in RIPC was first conceived with the identification that a period of reperfusion was mandatory in the remote organ to produce cardioprotection in rat experiments (42). The demonstration by Dickson *et al.* (45) that

blood taken from a rabbit, with simultaneous preconditioning of heart and kidneys, if transfused to an untreated rabbit would reduce infarct size in the untreated rabbit was further evidence for this mechanism. They also showed that coronary effluent from a preconditioned rabbit heart was also capable of reducing infarct size and improving LV function in the untreated rabbit heart (46). One must note that these experiments had preconditioned hearts and not just preconditioned remote organ, and therefore it does not give a firm basis of the humoral factor coming from a distal organ, but rather a role in the heart only. However, a pig model study by Konstantinov *et al.* (47) in 2005, that showed RIPC of the limb in pigs reduced infarct size in their transplanted hearts, strongly supports the role of humoral factor as the only possible way the signal relay could be possible. Recently Shimizu *et al.* (48) in their Langendorff heart model, have shown that this likely humoral factor is less than 15 kDa and is hydrophobic. Previously, Serejo *et al.* (49) in their rat model involving coronary effluent, had identified that this factor was larger than 3.5 kDa, and confers cardioprotection by activation of PKC. Therefore, it appears that this unknown humoral relay factor is between 3.5 to 15 kDa. Numerous studies have evaluated various endogenous autocoids and factors that are released in the remote organ. Some of these substances travel to the heart and exert their effect directly to the myocardium, and some also activate afferent neural pathways within the remote preconditioned organ to confer cardioprotection.

The endogenous substances implicated in this mediation include opioids, endocannabinoids and other receptor ligands. Patel *et al* (50) have shown that non-specific opioid receptor antagonist Naloxone, abolishes the cardioprotective effect of RIPC in intact rat model studies. Both δ1 and κ opioid receptors have been implicated in RIPC, however, there is some conflicting evidence regarding δ1 receptor involvement (51, 52). Opioids are thought to be transported and directly act on myocardium as cardioprotective substances. The endocannabinoid receptor CB2 has also been implicated in RIPC mediation. A recent rat model experiment has shown that CB2 blocker prevented the cardioprotective effect of intestinal ischaemia in infarct limitation (53). Endocannabinoids are also thought to be directly acting on the myocardium through CB2 receptors. Other receptor ligands which have been implicated include angiotensin receptor ligands and noradrenaline (54, 55).

1.5.1.2 Neural Mechanism

As discussed earlier, the first mechanistic insight of possible neural mechanism was shown by Gho *et al.* (42) in their rat model, where ganglion blocker, hexamethonium, abolished cardioprotective effect. Later, this effect was reproduced by many researchers using hexamethonium. Ding *et al.* (56) in their rabbit model of renal RIPC showed that sectioning renal nerve abolished cardioprotective effect. Also, they implicated the role of adenosine in activating renal afferents, showing that an adenosine blocker also abolished cardioprotection, and also decreased the renal afferent nerve discharge rates. The role of adenosine in RIPC was first implicated in 1998 in our own laboratory. We showed abolishment of the cardioprotective effect in rabbits treated with the adenosine receptor antagonist 8-sulphophenyltheophylline (8-SPT), prior to renal remote preconditioning (57). Further evidence of adenosine's role in a neural relay mechanism in RIPC was also shown by Liem *et al.* (58) involving mesenteric afferents. Dong *et al.* (59) in their rat hind limb remote ischaemic model, showed that severing femoral nerve abolished the myocardial protection of reducing infarct size conferred by limb RIPC, and further femoral artery adenosine injection decreased the infarct size. This has strengthened the necessity of neural integrity and adenosine's role in RIPC relay mechanism.

Bradykinin and Calcitonin gene related peptide (CGRP) are the two other endogenous substances implicated in neural pathway of RIPC, in which they act to stimulate the afferent nerve fibres from the remote organ. The role of bradykinin in RIPC was shown in an experiment involving mesenteric artery occlusion in male rats by Schoemaker *et al.* (60). They showed that bradykin B2 receptor blocker HOE140 abolished the myocardial protective effect. In addition, they showed that intra-mesentric bradykinin injection was also cardioprotective, and this effect was blocked by hexamethonium, suggesting that bradykinin acted through neural afferent stimulation. Wolfrum *et al.* (61) further showed that myocardial PKC-ε activation which happened with intestinal ischaemia was suppressed by B2 blocker HOE140 in a similar model, implicating bradykinins role prior to PKC activation.

The role of the neurotransmitter, CGRP, which is released from capsaicin-sensitive nerve terminals, is implicated in few experimental studies for its potential role in RIPC relay mechanism. Tang *et al.* (62) in their rabbit intestinal remote ischaemia model showed that pre-administration of capsaicin which prevents CGRP release, abrogates the cardioprotective

infarct reduction. Xiao *et al.* (63) reproduced similar effects in rat intestine models, and also showed that the abrogation of cardioprotective effect occurred with both capsaicin and L-nitroarginine methyl ester (L-NAME), an inhibitor of NO synthetase, suggesting that CGRP effect acts via nitric oxide pathway. Wolfrum *et al.* (64) showed in their intestinal remote preconditioning model, that CGRP release was associated with RIPC cardioprotection and appeared to act via PKC-ε.

1.5.2 Cardioprotection from Skeletal Muscle Ischaemia

Skeletal muscle remote preconditioning is of particular interest in this discussion as our studies (to be discussed in detailed in chapters 3 and 4) are based on this form of RIPC. Cardioprotection from skeletal muscle ischaemia was first shown by Birnbaum *et al.* (65) in their rabbit studies, involving partial occlusion of femoral artery blood flow. Instead of total occlusion, they used 30 minutes of partial occlusion of femoral artery blood flow to 55-60%. However, they induced demand-supply imbalance by rapidly pacing the gastrocnemius muscle. This maintained continuous perfusion yet providing ischaemia and allowed for facilitated transport of any humoral factors or mediators out of ischaemic zone. They showed reduction of 65% infarct size in this model. Subsequently, Oxman *et al.* (54) in their rat model with *in vivo* hind limb ischaemia, showed reduction in reperfusion arrhythmias in isolated Langendorff perfused hearts of rats, which underwent prior hind limb preconditioning. They also showed that this effect was due to increased release of norepinephrine in the perfused hearts, and postulated that norepinephrine was one of the humoral factors responsible for RIPC. Weinbrenner *et al.* (66) in their rat model of limb ischaemia by infra renal aortic occlusion, showed that reduction in myocardial infarct size was proportional to the duration of remote ischaemia, with 15 minutes of occlusion proving to be as protective as classic preconditioning with a reduction of infarct size to 18% compared to the control hearts which had infarct size of 52%. Ten minute and 5 minute remote ischaemic stimulus reduced infarct sizes to 37% and 42% respectively. They also showed that this protective effect was PKC dependent by showing the blockage of protection using chelerythrine, a selective PK C-inhibitor. In their experiment, hexamethonium did not block the protective effect, and this led them to conclude that the neural mechanism is unlikely to be involved in the signaling mechanism of RIPC.

The cardioprotective effect of skeletal muscle RIPC was also shown by Kharbanda *et al.* (67) in their pig model, where 4 cycles of 5-minute ischaemia followed by 5-minute reperfusion reduced experimentally induced myocardial infarction size. This study also showed that reduction of ejection fraction, which occurred during experimental myocardial infarction, was reduced in remote preconditioned pigs compared to controls. The same group also demonstrated the cardioprotective effect of lower limb RIPC in pigs undergoing cardiopulmonary bypass (CPB) with 2 hour cardioplegic arrest. They showed that 4 cycles of 5-minute lower limb ischaemia could reduce myocardial injury (Troponin release), lactate concentration (suggestive of tissue hypoxia), and acute lung injury (pulmonary resistance) (68). These benefits in systemic protection, not necessarily restricted to cardioprotection, in this study, was a vital signal of its wide beneficial effect to be found in clinical studies.

In view of its simplicity and noninvasiveness, transient limb ischaemia for providing RIPC is very attractive in human subjects. MacAllister's group was the first to characterise a transient limb ischaemia model in normal human volunteers (67). In their clinical study, Kharbanda *et al.* (67) induced transient limb ischaemia by applying a blood pressure cuff to the upper arm and inflating it to 200mmHg. Transient ischaemia was given for 5 minutes by cuff inflation, followed by reperfusion for 5 minutes through cuff deflation, a cycle repeated 3 times in the treatment group. They showed that the endothelial dysfunction for a 20 minute sustained ischaemia in the contra lateral arm was much lesser in remote preconditioned groups, as compared to controls. This was assessed through venous plethysmography for flow-response to acetylcholine, 15 minutes after sustained ischaemia. Loukogeorgakis *et al.* (69) characterised the time course of this remote preconditioning effect on endothelial protection of human volunteers. They showed that the endothelial protective effect measured by flow mediated dilatation response occurred immediately, however did not occur at 4 hours. The protection reappeared at 24 and 48 hours. This is an important study demonstrating in humans, the early and late phases of RIPC as seen in animal models. They also demonstrated that this protection was blocked by injection of trimetaphan (an autonomic ganglion locker), suggesting the role of neural mechanism in human RIPC.

1.5.3 Post Signal Relay Cardioprotective Mechanisms

Once the cardioprotective signal reaches the myocardium from the remote organ, intracellular signal transduction mechanisms are recruited within the cardiomyocytes, which are similar to those that participate in IPC and IPostC. These include G-protein cell surface receptors with their ligands such as adenosine, bradykinin, opioids, angiotensin and endocannabinoids (70). The binding of these cell surface receptors would then activate intracellular kinases, reactive oxygen species (ROS), nitric oxide, and mitochondrial K_{ATP} channel, which act as mediators of cardioprotection in the same way as described in IPC mechanism. It probably is better to highlight the commonalities between myocardial signaling response to IPC and RIPC involving the above mediators.

1.5.3.1 K_{ATP} Channel

Several studies have linked the opening of K_{ATP} channels in cardioprotection from remote ischaemic preconditioning. Treatment with K_{ATP} blockers like glibenclamide or 5-hydroxydecanate abrogated the remote preconditioning cardioprotection in these animal models (47, 57). Also, in a similar study Kristiansen *et al.* (71) have shown that this K_{ATP} channel appears to be mitochondrial, and not sarcolemmal K_{ATP} channel, in their rat model experiment. In the setting of IPC, mitochondrial K_{ATP} channel opening leads to mitochondrial ROS generation, which then mediates cardioprotection by either activating survival kinases or by inhibiting mPTP. This was discussed in earlier section. Whether this is also the case in RIPC is still not known.

1.5.3.2 Nitric Oxide

Nitric oxide (NO) generated through nitric oxide synthase (NOS), is an important mediator in the cardioprotective signaling cascade of IPC. In RIPC studies, there are conflicting data in its exact role in early RIPC, however, there appears to be a significant role of NO in delayed RIPC. In rat intestinal ischaemia model, Xiao *et al.* (63) had shown that delayed RIPC cardioprotection is abolished by nitric oxidase synthase inhibitor L-NAME. However, a similar experiment by Petrishchev *et al.* (72) involving early RIPC showed that nitric oxide blocker N omega-nitro-L-arginine (L-NNA) did not abrogate myocardial protection from RIPC. Shahid *et al.* (73) in their recent study in rat models have shown that cardioprotection

from RIPC is mediated by NO, and have also shown that NO is working upstream and acts via activation of mito-K_{ATP} channels, which subsequently increases the production of ROS. An experiment by Chen *et al.* (74) on Wistar rats also implicated the role of NO in early RIPC, as L-NAME did abolish the cardioprotective effect of RIPC in their model. Tokuno *et al.* (75) in their brain ischaemia model evaluating delayed RIPC cardioprotective effects, also implicated the NO role by showing abolished protection in iNOS knockout mice. When all studies are taken into account, it appears that NO plays a major role in signal transduction in both early and delayed RIPC.

1.5.3.3 PKC-epsilon (ε)

The role of PKC-ε in RIPC is implicated in many studies, which have shown that non-specific PKC blocker-chelerythrine abolishes the cardioprotection from RIPC stimulus (61, 66). It was also shown that the previously described mediators, bradykinin B2 receptor and CGRP cardioprotect via PKC-ε activation (61, 64). PKC-ε role in IPC is well recognised for opening K_{ATP} channels and subsequent ROS production, which leads to mitochondrial swelling (as discussed earlier). A similar role of PKC-ε in RIPC is suspected, however, this needs to be shown.

1.5.3.4 Reactive Oxygen Species (ROS)

ROS generation in a PKC-dependent manner and its role in activation pro-survival kinases (Akt and ERK1/2) in the IPC setting were discussed earlier. ROS involvement in RIPC cardioprotection was shown by Weinberenner *et al.* (52), in their rat model. They showed that the cardioprotection from RIPC was lost by administration of free radical scavenger. Whether ROS serves similar function of RISK pathway activation, and if it is involved in mPTP inhibition as in IPC is still not known.

1.5.3.5 RISK Pathway And Mitochondrial Permeability Transistion Pore (mPTP)

The RISK pathway refers to a group of pro-survival kinases which act downstream to PKC and they get activated at the time of reperfusion in the setting of IPC. The RISK pathway confers cardioprotection by inhibiting mPTP opening, anti-apoptotic signaling and anti-autophagy properties (21). The main components of the RISK pathway include Akt, ERK ½, PKG, p70s6K, and GSK-3β (21). There are also other kinases, which are thought to be pro-survival, however, it is not still very clear if they are truly protective or not. These kinases

include MAPK, P38 and JNK (21). RISK pathway activation is the final common pathway recruited in both IPC and IPostC (76), and therefore it is likely that RISK plays an important role in RIPC as well. One study has shown the increased phosphorylation of ERK1/2 in RIPC (77) setting and also have shown that an ERK1/2 antagonist would abrogate the cardioprotective effect of RIPC. This requires more studies to firmly establish the role of RISK in RIPC.

<u>1.5.3.6 Mitochondrial Permeability Transition Pore -mPTP</u>

The mitochondrial permeability transition pore (mPTP) has been the center of a growing amount of attention for its role in the mediation of reperfusion injury. The mPTP is a non-specific high conductance channel in the inner mitochondrial membrane, the formation of which leads to high permeability states with subsequent cell death (76). The inhibition of the mPTP by IPC and IPostC is well established, and the relevant mechanisms and studies are reviewed by Hausenloy *et al.* (76). The mPTP as an end effector in RIPC is however still not very firmly established. Zhang *et al.* (51) in their remote ischaemia model, have shown that the cardioprotective effect of RIPC was blocked by both κ-opioid receptor blocker and mPTP opener, indirectly suggesting the role of mPTP in RIPC cardioprotection. Further studies are needed to clarify mPTP inhibition in the setting of RIPC.

1.6 Clinical Application of Remote Ischaemic Preconditioning

The cardioprotective effect of RIPC seen in animal models of myocardial ischaemic reperfusion injury certainly is of huge interest for its applicability in human clinical settings, involving ischaemia reperfusion injury. As discussed earlier, MacAllister's group in 2002 (67) demonstrated its applicability in human volunteers, with beneficial effect on vascular endothelium to a prolonged ischaemic injury through remote preconditioning the contra-lateral arm. This meant that in clinical situations involving potential ischaemia reperfusion injury, this non-invasive intervention could be promising. Consequently, many settings exposed to IR injury involving various organs are being evaluated in clinical trials for the beneficial effect from RIPC. Cardiac surgery settings and myocardial revascularisation settings (surgical and percutaneous) have been evaluated in randomised trials so far, and many trials are underway in other non-cardiac settings involving IR injury. The first of the

studies which showed benefit of RIPC in clinical settings was done in the paediatric patient group undergoing corrective cardiac surgery (78). Subsequently, our institute in a randomised controlled trial involving 57 adult patients undergoing cardiac bypass surgery, showed a 43% reduction in peri-operative troponin-T release in remote limb preconditioned patients (79). Ali *et al.* (80) have shown a reduction in both myocardial injury and renal injury in the remote limb preconditioned patients undergoing elective abdominal aortic aneurysm repair.

1.7 Cardiac Revascularisation Associated Myocardial Injury

Both forms of revascularisations (PCI and CABG) are vital for treating coronary artery disease. However, both these forms of revascularisation can have a deleterious effect on the myocardium while its vascular supply is re-established. The mechanisms of this particular type of injury are different in settings of PCI and CABG. Consequently, the myocardial injury sustained during PCI (Peri-procedural myocardial injury) and during CABG (Peri-operative myocardial injury), will be discussed separately in detail.

1.8 Peri-Procedural Myocardial Injury During PCI

Although medical therapy can be used for prognostic and symptomatic benefit, coronary revascularisation, by either coronary artery bypass graft (CABG) surgery or percutaneous coronary intervention (PCI), remain important approaches for the treatment of occlusive CAD. In Europe, the number of PCI's increased from 184 000 to 885 000 during the period 1992 to 2004, with a projected increase to 1.5 million procedures by 2010 (81). In the USA, 1.3 million PCI procedures were performed in 2006 (82). Changing patient demographics, mainly linked to increased life expectancy, have resulted in the increased prevalence and complexity of CAD affecting an older age group.

Since the first description of coronary angioplasty by Andreas Grüntzig in 1977, the procedure has been extensively modified. The technical advances coupled with the use of coronary stents and adjuvant drug therapy, have resulted in high procedural success rates and low re-stenosis rates. Older patients are now being treated, and more complex multiple

coronary lesions deemed appropriate for PCI. PCI improves symptoms in patients with stable CHD, but does not improve clinical outcomes (83). About one third of all elective PCI procedures are associated with significant myocardial injury (termed peri-procedural myocardial injury, PMI), which has been associated with increased subsequent mortality (84). This underscores the importance of risk stratifying prior to the procedure to identify the patient group most likely to develop PMI. If PMI incidence can be reduced, clinical outcomes would be expected to improve. In this section, we review the underlying etiological factors resulting in PMI, the methods available for its detection and quantification, its prognostic significance, and the treatment strategies currently available and emerging designed to reduce this injury.

1.8.1 Incidence And Prognostic Significance Of Peri-Procedural Myocardial Injury

Cardiac biomarkers have been extensively used in the last two decades to establish the incidence and the prognostic implication of PMI. For the most part, these studies have focused on patients with stable CAD undergoing planned PCI, although patients with unstable CAD undergoing urgent PCI have also been included in some studies. The choice of cardiac enzyme assay, the proportional increase in the enzyme level, the criteria of enzyme cut-off value used to define PMI, and the timing and frequency of blood analysis can all affect the incidence of the PMI in studies evaluating cardiac biomarkers.

1.8.1.1 CK-MB

Although controversies regarding the prognostic significance of individual cardiac biomarkers persist, CK-MB elevation is widely accepted as a biomarker with prognostic significance when raised post-PCI. Elevation of CK-MB above the normal levels occurs in about 30% of patients undergoing elective PCI (84). More than 60 studies in the last two decades have assessed the prognostic significance of elevations in total CK and/or CK-MB fraction following PCI. Most meta-analyses that have analysed the majority of the prospective randomised studies, and few PCI registry studies (85-89) have inferred the proportionate increase in the early and late mortality with increased CK-MB release peri-procedurally (84, 90, 91).

1.8.1.2 TROPONINS

Troponins (Troponin I and Troponin T) are more sensitive and more specific markers of cardiac injury than CK-MB (92, 93). Troponin increases following PCI had originally thought to carry less prognostic importance than CK-MB elevations, although recent studies and meta-analyses have shown that Troponin elevations post-PCI are prognostically significant. Nienhuis *et al.* (94) in their meta-analysis of 15,581 patients from 20 studies over a 19 year period, reported the incidence of Troponin release post-PCI in elective PCI to be 33.0%, and increased mortality was significantly associated with Troponin elevation after PCI (4.4% vs. 3.3%, P = 0.001; OR 1.35). New generation high sensitive troponins (hsTrop), which have only recently become available, deserve attention at this stage. Although, studies looking at hsTrop elevation in the context of PCI are limited, there are a few studies looking at the incidence of this biomarker elevation in suspected ACS. In one study involving geriatric group of patients, high sensitive troponins were elevated in 76% of suspected ACS patients, but two thirds of these patients had no coronary disease in the angiography (95). A similar study involving 1026 patients comparing conventional troponin and high sensitive troponin assays reported higher diagnostic accuracy for detecting MI, but 51% were false positives (96). The cut-off value to define myocardial infarction in the context of PCI, therefore, is felt by many researchers to need re-assessment when using this biomarker

1.8.1.3 New Definition Of Peri-Procedural MI During PCI

The Joint ESC/ACCF/AHA/WHF Task Force Universal definition of Myocardial Infarction 2007(97) recently defined peri-procedural myocardial injury during PCI as an elevation of serum biomarkers (preferably cardiac Troponins) above the 99th percentile upper reference limit (URL) after PCI, assuming a normal baseline Troponin value. According to these published guidelines, an elevation in serum cardiac enzyme to more than three times the 99th percentile URL has been defined as a Type 4a PCI-related myocardial infarction. Applying the new definition of peri-procedural MI to the existing studies, Testa *et al.* (98) in their recent meta-analysis of 15 studies incorporating 7578 patients, observed that 15% of patients met the new criteria for peri-procedural myocardial infarction, and these patients are at high risk of further adverse events both during the hospital stay and at 18 months.

Based on the above findings, it appears that a routine Troponin assessment post-PCI may allow the identification of patients at risk of developing immediate and longer-term adverse events. However, the threshold level of troponin raise used to define periprocedural MI has generated lot of debate recently. A recent prospective study by Banning *et al* in which thirty two patients undergoing multivessel PCI and late gadolinium enhancement (LGE) cardiac magnetic resonance (CMR) had cardiac troponin I, CK-MB, and inflammatory markers (C-reactive protein, serum amyloid A, myeloperoxidase, tumor necrosis factor alpha) measured at baseline, 1 h, 6 h, 12 h, and 24 h after the procedure.(99) Three "periprocedural injury" groups were defined with the universal definition: G1: no injury (biomarker <99th percentile); G2: periprocedural necrosis (1 to 3 × 99th percentile); G3: myocardial infarction (MI) type 4a (>3 × 99th percentile). Differences in inflammatory profiles were analyzed. With CK-MB there were 17, 10, and 5 patients in groups 1, 2, and 3, respectively. Patients with CK-MB–defined MI type 4a closely approximated patients with new CMR-LGE injury. Groups defined with CK-MB showed progressively increasing percentage change in C-reactive protein and serum amyloid A, reflecting increasing inflammatory response (p < 0.05). Using cardiac troponin I resulted in 26 patients defined as MI type 4a, but only a small minority had evidence of abnormality on CMR-LGE, and only 3 patients were defined as necrosis. No differences in inflammatory response were evident when groups were defined with troponin. The study concluded that Measuring CK-MB is more clinically relevant for diagnosing MI type 4a, when applying the universal definition. Current troponin thresholds are oversensitive with the arbitrary limit of 3 × 99th percentile failing to discriminate between periprocedural necrosis and MI type 4a. Therefore the meaningfulness of using troponins as a sole outcome measure in the research might be a pitfall in itself.

1.8.1.4 ECG Changes

Although a 12-lead surface ECG is continuously recorded during the cardiac catheterisation procedure, the sensitivity of the ECG for detecting ischaemia during PCI has been disappointing. Unipolar intra coronary ECG recording appears to be more sensitive and

reliable in detection of ischaemia during PCI (100, 101). However, intra-coronary ECG has not been generally adopted during PCI, its role being relegated to historical interest only.

1.8.1.5 Cardiac MRI

Non-invasive imaging of the heart by cardiac magnetic resonance (CMR) imaging can be used to detect the location of the PMI and quantify its extent. The presence and extent of increased signal intensity on late gadolinium enhancement CMR can be used to image myocardial injury directly following PCI, with minimal inter-observer and intra-observer variability (102-104). In a prospective study involving 50 patients, Selvanayagam and co-workers (105) showed a very strong correlation with CMR defined new myocardial infarction and elevated Troponins post-PCI, thereby validating Troponin release post-PCI as a marker of myocardial necrosis.

1.8.2 Mechanisms of Myocardial Injury During PCI

The most common mechanisms of myocardial injury during PCI are distal embolisation and side-branch occlusion (SBO). Other significant causes include dissection, thrombus, no reflow/slow flow, or coronary perforation. Herrmann J in his review on PMI in 2005 (106), classified PMI into two types. Type 1 (Proximal type), which is in proximity to the target lesion of PCI and may be due to side-branch occlusion, and Type 2 (distal type) that is in the perfusion territory of the treated coronary artery, and mainly due to structural and functional microvascular obstruction (106). Fifty to 75% of all PMI is Type 2 (the distal type) (106).

1.8.2.1 Type 1 or Proximal Type of PMI

Proximal type of PMI is most often seen adjacent to the treated arterial segment. This type of myocardial injury is mainly attributed to side-branch occlusion (SBO), which can happen during balloon inflation or with stent insertion. Side-branches are within the vicinity of the angioplasty site in over 50% of cases and, although many are unaffected, a considerable

proportion are compromised by the procedure (107). Occlusion of a side-branch has been reported in up to 19% of cases in which a stent was placed across a major side-branch (>1mm) (108). Most occlusions occur after post-stent dilation performed with high-pressure inflations (108).

Side-branches originating from within the lesion of the native coronary artery are at a higher risk of occlusion during PCI and, if ostial disease is present in the branch vessel, there is a five- to ten-fold increase in the possibility of side-branch compromise (108-111). Other influencing factors include the branch relationship to parent vessel lesion, branch vessel size, and balloon to artery ratio (108-111). The proposed mechanisms of SBO include snow plough effect (plaque shift), thrombus formation, dissection of the dilated artery involving the take-off of the side-branch, side-branch spasm and plaque embolisation (109, 110, 112).

1.8.2.2 Type 2 or Distal Type of PMI

This type of myocardial injury is seen in the distal perfusion territory of the treated epicardial artery and accounts for 50 to 75% of PMI (106). The atherosclerotic plaque disruption and local vessel trauma are the predominant cause in the distal injury and possess occlusion potential of both epicardial vessels and myocardial microvascular levels (106). The potential mechanisms through which this disrupted plaque and local trauma leads to distal perfusion territory insult include:

a. Distal embolism of atheromatous debris and thrombotic debris.
b. Platelet activation and thrombosis, leading to micro-vascular plugging of platelets and neutrophils.
c. Neuro-hormonal activation and modulation of vascular and myocardial functions.
d. Oxidative stress and inflammation

1.8.2.2.1 Distal Embolism of Atheromatous Debris and Thrombotic Elements

Distal embolisation as a result of plaque denudation during percutaneous trans-luminal angioplasty (PTCA), was first described in the 1980s (113). Recent developments in intravascular ultrasound (IVUS) techniques have contributed to the understanding of

atherosclerotic plaque morphology and its components (114). Compared to balloon angioplasty, in which the lumen expansion is predominantly due to plaque redistribution and plaque dissection, lumen enlargement after stenting involves a combination of plaque redistribution, plaque extrusion, vessel expansion, plaque compression, and plaque embolisation (115, 116).

Using filter devices distal to the lesion, studies have identified that the embolisation of plaque fragments frequently occurs during PCI (117, 118). The atherosclerotic plaque burden in the lesion before intervention is correlated with increased PMI as evidenced by subsequent cardiac enzyme elevation (119). The development of IVUS Virtual histology (VH) has enabled, *in vivo* identification of the histopathological characteristics of plaques, and the usage of Doppler wire and evaluating high intensity signals (HITS) enables direct detection of small embolic particles which could not be detected with conventional angiography (120). Using these techniques, Kawamoto *et al.* (120) have shown the impact of distal emboli and plaque characteristics on coronary microcirculation, as assessed by Coronary Flow Velocity Reserve (CFVR) during PCI, in a study involving 44 patients with stable angina. Patients in the highest tertile of HITS had a significantly larger necrotic core area compared with patients in lower tertiles. In addition, there was a small but significant negative correlation between HITS and CFVR after PCI.

Atherosclerotic plaque of the lesion, which have larger necrotic core (NC) are at higher risk of plaque rupture and micro-embolisation during PCI and subsequent cardiac enzyme release (114, 120). The necrotic core component contains fragile tissues, such as lipid deposition with foam cells, intramural bleeding, and/or cholesterol crystals, and these tissues are often separated from the vessel lumen by only a thin fibrous cap and, hence, they are thought to be easily liberated as small emboli during coronary stenting (120-122).

1.8.2.2.2 Platelet Activation and Thrombosis- leading to Microvascular Plugging of Platelets and Neutrophils.

During PCI plaque rupture, the arterial endothelial barrier is denuded, and atherosclerotic material, connective tissue elements, and sub-endothelial matrix proteins (collagen, von Willebrand factor) are exposed to blood (123). Platelets adhere to collagen and von

Willebrand factor via specific cell receptors (glycoprotein [GP] VI, GP Ia/IIa, GP Ib-IX), and become activated (124, 125). Activated platelets de-granulate and secrete agonists, chemotaxins, clotting factors, and vasoconstrictors that promote platelet aggregation, thrombin generation, and vasospasm.

Increased platelet aggregation during PTCA was shown in 1993 by Gasperitti *et al.* (126), in the blood drawn of coronary sinus during angioplasty. In a recent study by Mahemuti *et al.* (127) a transient significant increase in TF (14%; P = 0.004), prothrombin fragments 1 and 2 (40%; P = 0.001), and F-VIIa (31%; P = 0.007) following angioplasty were reported, although the levels returned to normal after stents were deployed. Cuisset *et al.* in 2007 (128), showed the higher incidence of PMI in low responders to dual antiplatelet therapy in a non-ST elevation acute coronary syndrome (NSTEACS) patient cohort. This was established using high post-treatment platelet reactivity (HPPR) (maximal intensity of ADP 10 μM-induced platelet aggregation >70%), which identifies low responders to dual antiplatelet therapy (aspirin and clopidogrel). PMI occurred significantly more frequently in patients with HPPR than in the normal-responders, as evidenced by post-procedure Troponin I release (43% vs. 24%, p=0.014). Previously, Chen *et al.* in 2004 (129), had shown that Aspirin-resistance is associated with a high incidence of myonecrosis after non-urgent PCI despite clopidogrel pre-treatment using rapid platelet function assay-ASA (RPFA-ASA). The incidence of any CK-MB elevation was 51.7% in Aspirin-resistant patients and 24.6% in Aspirin-sensitive patients (p < 0.006). Elevation of cTnI was observed in 65.5% of Aspirin-resistant patients and 38.5% of Aspirin-sensitive patients (p < 0.012).

The release of potent bio-factors like tissue factor (TF) leading to micro-vascular thrombosis and no-reflow during *in vivo* plaque disruption by PTCA was demonstrated by Bonderman *et al.* (130) in 2002.

Mizuno *et al.* (131) in 2000, showed increased levels of coagulation factors in coronary circulation after PTCA despite adequate administration of intravenous heparin. TF levels in coronary sinus blood were elevated 4 hours after PTCA, followed by increased levels of thrombin-antithrombin III complex, a specific and sensitive marker for thrombin generation, 24 hours after PTCA. It was further confirmed by Salloum *et al.* in 2005 (132), that a significant amount of TF is released *in situ* immediately after PCI in saphenous venous grafts (SVGs), measured by aspiration through export catheter.

1.8.2.2.3 Neuro-hormonal Activation and Modulation of Vascular and Myocardial Function.

Coronary vasospasm distal to the PTCA site was shown in arteriographic analysis in 1988 by Fischell TA *et al.* (133). Microcirculatory vasospasm is thought to be an important phenomenon, occurring during coronary interventions. Potent vasoconstrictors, like serotonin (5-HT) and endothelin, which are released by activated platelets, were shown to be significantly increased in the distal coronary bed during SVG PCI (132).

Microcirculatory vasospasm is thought to play a significant role in the 'No reflow phenomenon' frequently seen during Primary PCI (134). The neural mechanism of vasoconstriction is supported by studies which have shown that alpha-adrenoreceptor blockade by drugs like uradipil and yohimbine, attenuates coronary vasoconstriction and increases coronary flow reserve (CFR) during coronary angioplasty and rotational atherectomy (135-137).

1.8.2.2.4 Oxidative Stress and Inflammation

Angioplasty associated increase in isoprostane -PG(F2)Alfa and ischaemia modified albumin (IMA), which are associated with free radical damage through reactive oxygen species (ROS) generation, suggests oxidative stress to be an important mechanism of PMI (138, 139). However, most of the studies on oxidative stress were carried on primary PCI patients, many of whom have completely occluded arteries, suggesting that oxidative stress in these conditions owes more to reperfusion injury.

Rises in inflammatory markers interleukin-6 and C-reactive protein (CRP) levels post angioplasty and PCI have been shown in clinical studies (140, 141). Bonz *et al.* in 2003(142), showed that the inflammatory markers were higher in patients who had significant Troponin release post PTCA, as compared to patients who had no Troponin release post-procedure, suggesting inflammation as one of the mechanisms of PMI. Gach *et al.* (143) in 2005, showed early increased release of neutrophil markers (myeloperoxidase, lactoferrin) in patients undergoing stents, which did not increase with diagnostic angiography.

1.8.3 Factors Influencing PMI

The key factors which influence the incidence and magnitude of PMI can be broadly classified into patient factors, angiographic or lesion related factors, and procedural factors. Assessment of these factors prior to the intervention allows risk stratification for PMI.

1.8.3.1 Patient Factors

Patient factors implicated for higher incidence of PMI include: older age (144), multi-vessel CAD (106), diffuse CAD (145), systemic atherosclerosis (145), pre-existing renal impairment (146), presence of anaemia (147), pre-procedural C reactive protein (CRP) elevation (148), and pre-procedural white blood cell count >9.5 x 10^6/L (149). Also, patients with evolving MIs with elevated cardiac enzymes before the procedure are at increased risk of PMI. This was shown in a sub-analysis from the IMPACT-II trial (89). In the setting of unstable angina and acute coronary syndromes, Troponin T elevation is associated with more complex coronary stenoses and an increased likelihood of multi-vessel CAD (150-152). The plaque seen in coronary arteries of acute coronary syndromes (ACS) have large lipid cores and a thin capsule, the so called 'vulnerable plaque', compared to coronary arteries in patients with stable angina, which appear to have a thick capsule (153). The unstable plaque in the setting of an ACS is more likely to denude during revascularisation procedures. In the non-ST elevation acute coronary syndrome (NSTEACS), this increased PMI with early intervention was shown in a recent trial of 1200 Troponin positive patients; ICTUS (Invasive Versus Conservative Treatment in Unstable Coronary Syndromes Investigators). The incidence of PMI was significantly higher in the early invasive strategy group than the selective invasive group (11.3 percent vs. 5.4 percent, P=0.001) (154).

In the case of Primary PCI, myocardial injury also occurs due to reperfusion injury with a mechanism distinct from that associated with peri-procedural injury (8). As both Reperfusion Injury and PMI are associated with cardiac enzyme rise, it is difficult to separate the relative contributions towards myocardial injury by these two processes. The cardiac enzyme released due to the infarction itself further complicates interpretation.

1.8.3.2 Angiographic or Lesion related Factors

Complexity of the arteriosclerotic lesions and coronary anatomy influence the amount of PMI, presumably due to prolonged and aggressive catheter manipulation. The plaque burden, number of lesions, presence of bifurcation lesions, tortuosity and calcification are all likely to influence myocardial injury during PCI. A recent study investigating the ability of four angiographic classification schemes to predict peri-procedural myocardial injury in patients undergoing PCI for stable angina, found the Syntax scoring system to be reliable in predicting PMI (155).

In addition, coronary embolisation of atherothrombotic debris, resulting in elevated cardiac enzymes and an ST-elevation or non-ST elevation MI during catheter intervention, is a more common problem after PCI in saphenous vein grafts (SVGs) rather than in native coronary arteries (156, 157). A possible explanation is that atherosclerotic plaques in vein grafts are softer, more friable, and are more commonly associated with thrombus and platelet activation compared to plaques in native coronary arteries, all these features making SVG lesions prone to fragmentation and distal embolisation during PCI (158, 159).

1.8.3.3 Procedural Factors

The incidence of PMI with elevation of cardiac enzymes is significantly higher in Directional Coronary atherectomy (DCA) compared to PTCA. (16% versus 6%; P<.0001)(160-162). Also, suboptimal stenting has been shown to be associated with increases in the peri-procedural incidence of non-ST elevation MI (NSTEMI); 8.7 versus 4.2 percent, p =0.003). In a recent study, Hoole *et al.* (163) also implicate longer stent length for increased myocardial enzyme release.

1.9 Peri-operative Myocardial Injury During CABG

Coronary artery bypass graft (CABG) surgery, which is the surgical way of myocardial revascularisation of the diseased coronary arteries, predates the percutaneous revascularisation. In fact, the development of coronary artery bypass technology in the 1950s spurred surgeons to develop coronary artery bypass grafting. Cardiac surgery developed later than other surgical specialties, largely due to technical difficulties of operating on the heart. This procedure has also seen tremendous advancement, both in the surgical aspects, choice of conduits and in the myocardial protection from peri-operative effects which will be discussed in detail here. Although PCI was devised 20 years later in 1970s, it has surpassed CABG in the number procedures performed annually. However, CABG still remains the most common cardiac surgery performed in the world.

Although, a lot of early operations attempted to operate on the surface of the heart, they were limited by the technology of their time. Most were very ingenious, and in many ways ahead of their time. These operations to treat CHD included methods to increase non coronary blood flow to the heart by creating pericardial adhesions (164, 165). Some also studied the effects of coronary sinus ligation in an attempt to impede venous outflow and thereby improve myocardial perfusion. However, in the final analysis they all required the ability to support the circulation to make the breakthrough that they were seeking. The crucial technology of artificial circulatory support was developed, principally by the perseverance of Dr John Gibbon (166). The "heart lung machine" of Gibbon could support circulation, and was a major breakthrough in the field of cardiac surgery. Also, in 1950 Bigelow had found that in experimental models the total body oxygen consumption was decreased with temperature, and this included myocardial metabolism (167). With the initial heart lung machines, there was need for high flow rates to provide for the body's oxygen demands. But this need was circumvented when investigators reassessed Bigelow's previous findings, and found that by adding hypothermia, the total body oxygen requirements were greatly reduced in patients.

The surgeons in 1960s reviewing complications of cardiac surgery did not initially consider the possibility of myocardial injury, due to surgery itself. It was in 1967, when Taber's group first reported that there was myocardial necrosis following cardiac surgery, and showed that

patchy necrosis up to 30% of the myocardium occurred post cardiac surgery (168). Cooley *et al.* (169) in 1972 reported the phenomenon of 'stone' heart, when the heart goes into a state of ischaemic contracture during double valve surgery, especially when the ischaemic time was prolonged. With many researchers finding similar peri-operative myocardial injury, the question of whether the protection of myocardium during cardiac surgery is adequate became serious, and improving protective techniques were explored.

1.9.1 Cardioplegia Use for Myocardial Preservation

The first use of 'elective cardiac arrest' was by Melrose in 1955, who also coined the term ''cardioplegia'' for the technique (170). Melrose used a solution containing potassium citrate to remove trans-membrane electrical potential, and to stop cardiac impulse and arrest the heart in diastole, by injecting the solution into aortic root. There was initial wide interest in cardioplegia however with experience there were evidences of late myocardial necroses following use of Melrose solution. This was shown to be due to extremely high concentrations of potassium and high osmolarity of Melrose solution (171). Cardioplegia, thus fell into disrepute until there was a better understanding of the components necessary for safely inducing electromechanical arrest, and preserving cell structure and function during ischaemia (172). The use of hypothermia with continuous coronary perfusion continued to be the predominant method, and this was supplemented with the use of ventricular fibrillation to induce cardiac standstill.

The resurgence in the interest in cardioplegia in 1970s could be attributable for pioneering work by Gay and Ebert who used crystalloid solution for cardioplegia which contained much lower concentrations of KCL (173). Hearse and his group's further work on potassium solutions led to the development of St Thomas's solution which was first used clinically in 1976 (174). Since then, there has been a lot of work done to enhance the substrate of cardioplegic solution by many researchers. The essential clinical prerequisites for cardioplegia include a solution that is safe in ischaemic models, the distribution of flow to all cardiac regions, periodic replenishment to counteract non-coronary collateral washout and strategies for protection in various clinical conditions (175). The likely composition of

current pharmacological cardioplegic solutions and principles are summarised in the table below.

Table 1

PRINCIPLE	METHOD
Immediate arrest	K^+, Mg^{2+}, procaine
Hypothermia	10˚C-20˚C
Substrate	Oxygen, glucose, glutamate, aspartate
Appropriate pH (buffer)	Tris-hydroxymethyl aminomethane(THAM) solution, bicarb, phosphate
Membrane stabilization	Ca^{2+}, steroids, procaine, calcium channel blockers, magnesium, O_2 radical scavenger

Reproduced from Allen BS, Buckberg GD Myocardial management of arterial Revascularization, 2006.(175)

Blood as a cardioplegic vehicle was first introduced by Dr Gerald Buckberg's group in 1978, after their vast experimental work (176). Blood cardioplegia has become most popular method of cardioplegia in the current era. Very few surgeons however continue to use crystalloid cardioplegia and other methods of myocardial protection, like fibrillatory arrest. Blood cardioplegia is the preferred choice, as this physiological source of oxygen is available readily in the extracorporeal circuit, and its use limits the haemodilution when large amounts of cardioplegia are needed. Also blood proteins like histidine aid in buffering capacity required for cardioplegia. Furthermore the rhelogic advantage on microvasculature afforded by the erythrocytes enhance papillary muscle perfusion compared to oxygenated crystalloid cardioplegia and reduce coronary vascular resistance and oedema formation (175). The erythrocytes in blood also contain many endogenous oxygen free radical scavengers (superoxide dismutase, catalase and glutathione) (175). The current practice in most surgical centres of the world is usage of cold blood cardioplegia. The cardioplegia is also used retrogradely through coronary sinus in many centres. Surgeons differ in their opinion in preferred cardioplegic route and some advocate a combination of antegrade and retrograde cardioplegia in during surgery. Antegrade cardioplegia is thought to not provide adequate perfusion to distal areas of complete coronary occlusions, where retrograde perfusion is of value, however retrograde cardioplegia is criticised for its inadequacy of right ventricular

perfusion. Consequently, in many centres a combination of the two, adapted to particular surgery and patient is usually practiced (177).

The other cardioprotective strategy that few surgeons use other than cardioplegic arrest, is by inducing electrical ventricular fibrillatory arrest. The argument for this strategy is that, the perfusion to myocardium remains intact until aortic cross clamp is applied and hence myocardium is exposed to lesser ischaemic burden. In this method, the proximal anastomosis to the aorta is created without the need of cross-clamping, and aortic cross clamp is applied intermittently when distal anastomoses are created. This, hence, reduces ischaemic time that the myocardium experiences significantly. However, it also means that the cardioplegic protection with oxygen and substrates provided by cardioplegia solution is deprived in this method. Potentially, hence, myocardium could experience more injury for given ischaemic time when compared to cardioplegia, although the conundrum is that the ischaemic time itself is reduced.

In the United Kingdom, a survey in 2004 indicated that among surgeons performing on-pump CABG, 85% used cardioplegia, mostly antegrade cold cardioplegia and 15% used cross clamp ventricular fibrillation as cardioprotection (178).

1.9.2 Need for Further Myocardial Protection.

Although considerable progress has been made in surgical techniques and other perioperative myocardial protective strategies, allowing for the majority of patients to undergo cardiac surgery without significant mortality, a significant proportion of patients may still experience substantial morbidity related to adverse cardiovascular events. These include prolonged contractile dysfunction (stunning), myocardial infarction, low-output syndromes, and overt ventricular failure, all resulting in prolonged intensive care unit stay and reduced functional capacity and ultimately contribute to overall mortality. Although the predominant mechanism for this myocardial injury still is ischaemia-reperfusion injury, there are other causes, which can affect myocardial injury sustained during cardiac surgery. These include the inflammatory response to extraneous substances in the cardiopulmonary bypass circuit, athero-embolism, over distension and direct myocardial injury due to retraction and handling of the heart (179). Generally, these latter influences are well understood and are minimised by intra-operative cardiac decompression, appropriate care

in handling and retracting the heart and aorta, as well as careful monitoring and manipulation of heart rate, filling pressure, and systemic vascular resistance (179). Also, delivery of cardioplegia solution at high infusion pressure can be deleterious to myocardium especially to ischaemic tissue (175). This is especially in cases of anterograde cardioplegia where perfusion pressure in the coronary arteries is not usually monitored.

1.9.3 Biomarker Elevation after CABG

It is not surprising that elevation of cardiac biomarkers occurs in most of the patients undergoing elective CABG. Cardiac Troponins (cTn I or cTn T) and CKMB are used for diagnosis of myocardial necrosis. The diagnostic accuracy of these biomarkers is reduced after CABG due to their release as a routine sequelae of the procedure (180, 181). The incidence of elevated CKMB above ULN has been noted in 62-90% of the patients undergoing CABG (180, 181). However, patients with substantial elevation of cardiac enzymes are known to have worse prognosis. In a review of 2918 patients, the six month mortality associated with elevations of CK-MB <5, ≥5 to ≤10, ≥10 to ≤20, and >20 times above the upper limit of normal was 3.4, 5.8, 7.8, and 20.2 percent, respectively; this relationship remained significant after adjusting for other risk factors (182). Also, in a series of 3812 patients, serum CK-MB was significantly predictive of increased mortality, only with values more than 10 times the upper limit of normal (180). This increase in mortality persisted at an average follow-up of three years. Likewise, the increase of Troponin levels after CABG indicates necrosis of myocardial cells, which predicts a poor outcome in particular when elevated to the highest quartile or quintile of the measurements (183, 184). The Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction has classified myocardial infarction associated with CABG surgery as 'Type 5 myocardial infarction'. Unlike the prognosis, scant literature exists concerning the use of biomarkers for defining myocardial infarction in the setting of CABG. Therefore by arbitrary convention, biomarker values more than five times the 99th percentile of the normal reference range during the first 72 h following CABG, when associated with the appearance of new pathological Q-waves or new LBBB, or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium, is considered as diagnostic of a CABG related myocardial infarction (type 5 myocardial infarction) (97).

1.9.4 Cardiac Imaging in Diagnosis of Perioperative Myocardial Injury

Apart from cardiac enzymes and ECG changes, imaging modalities can also be used to determine the occurrence of myocardial injury and also can localise the injured myocardium. Myocardial SPECT and cardiac MRI are the two imaging methods, which have been used to assess perioperative myocardial injury. Using contrast enhanced magnetic resonance imaging; Steuer *et al.* (185) in their study of patients undergoing CABG have demonstrated the visualisation and the quantification of myocardial necrosis post CABG. They also showed strong correlation with the Troponin T release and CKMB release, and MRI findings of myocardial necrosis. There was strong inter-observer agreement in quantification of infarct mass through MRI in this study. The perioperative myocardial injury using SPECT scan was demonstrated as early as 1989 by Burns *et al.* (186). The correlation of SPECT imaging infarct size with enzymatic and other modalities of quantification of infarction is also well established (187). Direct comparison between the two modalities on detection and reproducibility of the infarct size have shown that cardiac MRI is more sensitive and better reproducible technique than SPECT sestamibi and also more sensitive in diagnosing subendocardial infarctions (188-190).

1.9.5 Factors Influencing Perioperative Cardiac Enzyme Release

There are many factors involving the surgical setting of CABG surgery, which can influence the myocardial injury and consequent enzyme release. Some of the known factors are discussed below.

a. Cardioplegia vs Cross Clamp Fibrillation

The type myocardial protective strategy that is used in CABG is likely to influence myocardial injury during CABG. The 'Cardioplegia' and 'cross clamp fibrillation' methods are the two commonly employed myocardial protection strategies. As discussed in the previous section, intermittent cross clamp fibrillation is likely to decrease the ischaemic time that the surgery requires, as the myocardial perfusion remains intact until the aortic clamps are applied.

However, it also means that the cardioplegic protection with oxygen and substrates provided by cardioplegia solution is deprived in this method. Therefore, potentially myocardium could experience more injury for given ischaemic time when compared to cardioplegia. A prospective randomized study by Musumeci *et al.* (191) involving 91 patients undergoing CABG, showed that intermittent cross clamp fibrillation (ICCF) was better in reducing perioperative cardiac enzyme release compared to cold blood cardioplegia method. The ischaemic time in the ICCF group was significantly lower so this could have influenced the results. A meta-analysis by Scarci *et al.* (192) involving 13 studies showed no difference in cardiac enzyme release between these two methods. No study to-date has evaluated the enzyme release by these methods as a ratio of ischaemic time. Majority of the surgeons across the world use cardioplegic method as their choice. In the United Kingdom, a survey in 2004 indicated that among surgeons performing on-pump CABG 85% use cardioplegia, mostly antegrade cold cardioplegia and 15% use cross clamp ventricular fibrillation as cardioprotection (193).

b. Blood Cardioplegia vs Crystalloid Cardioplegia

Being a physiological buffer with oxygen carrying capacity, blood cardioplegia is now the preferred choice of cardioplegia and the advantages of using blood cardioplegia were discussed in an earlier section. In terms of myocardial enzyme release, two meta-analyses evaluating a total of 52 randomised trials have concluded that blood cardioplegia results in significantly less myocardial enzyme release compared to crystalloid cardioplegia (194, 195). However, there are still many surgeons across the world committed to crystalloid cardioplegia. The likely advantages of crystalloid cardioplegia are it is cheaper and quicker to administrate and gives a better view when performing distal coronary artery anastomoses (194).

c. On Pump vs Off Pump CABG

In the last decade, less invasive cardiac operative techniques have emerged and off-pump CABG (OPCAB) is currently most widely practiced minimally invasive surgery. This involves bypass grafting on a beating heart and avoids usage of cardiopulmonary bypass. The ischaemia to heart during the operation is only regional, as compared to global ischaemia in

conventional CABG, as individual coronary arteries are clamped separately during individual vessel grafting. It is logical to imagine that this would reduce myocardial injury and outcomes, however large randomised studies and meta-analysis have shown no benefit in mortality, morbidity or incidence of peri-operative MI (196, 197). Although the ischaemia is regional, the relative hypoxic injury in OPCAB myocardium is likely to be more than that in conventional CABG where the heart is cardioplegic and hence with lesser oxygen demand during ischaemic period.

d. Ischaemic Times and Cardiopulmonary Bypass Times (CPB)-

The ischaemic time refers to the duration of time the myocardium is deprived of vascular perfusion during CABG. This corresponds to aortic cross clamp time during on pump cardiac surgeries, and in OPCAB surgeries, it corresponds to the total coronary artery clamping times. It is very logical to expect that, higher the myocardial ischaemia duration, correspondingly more myocardial injury to be expected as many areas of myocardium enter from reversible into irreversible injury phase. A cross clamp time of more than 90 minutes is shown to be an independent predictor of significant perioperative myocardial injury (198). Prolonged cross clamp times and CPB time often denoted technical difficulties, in executing the planned operation. This could be due to unfavorable anatomy, unexpected intra-operative complications, or difficulties in weaning the patient from bypass, may be due to residual ischaemia or post-CPB myocardial stunning, all of which are factors responsible for cardiac enzyme elevation. A CPB time of more than 120 minutes is shown to be independent predictor of significant perioperative myocardial injury (199). One study has shown that the cardiac enzyme release at the end of the procedure correlates to both aortic cross clamp times and CPB times (200).

1.10 Current and Emerging Therapeutic Strategies to Reduce Revascularisation Associated Myocardial Injury

Both peri-procedural myocardial injury during PCI, and peri-operative myocardial injury during CABG carry well known prognostic implications in terms of both mortality and morbidity, as already discussed in their relevant sections. The search for preventing and reducing these forms of ischaemia-reperfusion injury, therefore, has a very important place

in the clinical field. Consequently, various therapeutic strategies have been explored over years with significant success in reduction of these forms of injuries. We shall now explore these strategies, in the individual settings of PCI and CABG separately, as the pharmacological strategies are different in these two settings although many therapies overlap.

1.10.1 Therapeutic Strategies to Protect from Peri-procedural Myocardial Injury

The therapeutic strategies being pursued to combat PMI can largely be divided into three subgroups.

A. Strategies to prevent side-branch occlusion
B. Strategies to prevent distal embolisation and microvascular coagulation.
C. Strategies of protecting the myocardium itself against PMI (Cardioprotection).

1.10.1.1 Strategies to Prevent Side-branch Occlusion.

Bifurcation lesions are always difficult to treat. Although strategies of stenting both main vessel and side-branch using drug eluting stents (DES) using different techniques like 'crush' and 'culotte' are evaluated, no long term benefits have been found in RCTs (201-203). In fact, in BBC-ONE trial there was a higher MI incidence including PMI in 'complex stent strategy' (203). A 'simple strategy' of main vessel PCI with an option of side-branch stenting or balloon, only if warranted appears to be the current preferred practice.

1.10.1.1 Strategies to Prevent Distal Embolisation and Microvascular Coagulation.

Antiplatelet and Anti-thrombotic Drugs.

Platelet activation plays a cardinal role in the pathophysiology of PMI. Anti-platelets and anti-thrombotics are used as first line preventative drugs to counteract the pro-coagulant milieu created during coronary interventions and hence to minimise myocardial damage.

Aspirin

Aspirin has been known to be protective against peri-procedural Q wave MI, for more than 20 years. However the initial trials involved very large doses of aspirin (e.g. 990mg) or aspirin combined with Dypiridamole or Ticlopidine. Protection against immediate PTCA complications when compared to placebo were shown by these trials (204, 205). There have been no randomised trials to determine optimum loading dose of Aspirin prior to PCI, but the guidelines suggest dosages of 75-325mg in patients already on long term low dose Aspirin, and 300 to 325mg Aspirin before PCI if not previously treated with Aspirin (206). The need for additional anti-platelet treatment in PCI is due to Aspirin being a comparatively weak inhibitor of platelet function. Other agonists such as ADP, collagen, or thrombin can still activate platelets, as measured by ex vivo tests in patients taking Aspirin (207). There also is an approximate 24% prevalence of Aspirin resistance in the community (208).

Thienopyridines

Thienopyridine drugs include the anti-platelet agents Clopidogrel, Ticlopidine and Prasugrel. The metabolites of these drugs irreversibly bind to ADP (P2Y12) receptors on the platelet, thus attenuating ADP-mediated GP IIb/IIIa receptor activation and platelet aggregation.(209) Clopidogrel has been adopted as the drug of choice over ticlopidine in view of enhanced safety and tolerance. However Clopidogrel resistance is common in the population. Following a 300-mg oral clopidogrel loading dose and 75 mg administered daily thereafter, 'resistance' was observed in 31% and in 15% of patients at 5 and at 30 days post-PCI, respectively (210). A higher loading dose of 600 mg clopidogrel was associated with a lower incidence of early resistance (8%) compared with a 300 mg load (25%)(211). The results from the ARMYDA 2 trial suggested that a 600-mg loading dose of clopidogrel safely and more effectively reduced the occurrence of peri-procedural infarctions than did a 300-mg loading dose (212). Multivariable analysis showed that pre-treatment with the 600-mg clopidogrel loading regimen was associated with an approximately 50% risk reduction of peri-procedural myocardial infarction (Odds ratio 0.48, $P = 0.044$). Prasugrel, a new thienopyridine has been found to have much less variability in response compared to clopidogrel and appears not to have non responders, however its role in PMI reduction is not yet established (213).

GPIIb/IIIa inhibitors

Platelets adhere to collagen and von-Willebrand factor via specific surface membrane glycoprotein cell receptors and become activated. The platelet GP IIb/IIIa receptor is of particular interest because of its central role in platelet aggregation. Three intravenous GP IIb/IIIa inhibitors are currently available for clinical use: abciximab, tirofiban and eptifibatide. Abciximab is a monoclonal antibody directed against the receptor, while tirofiban and eptifibatide are high affinity non-antibody receptor inhibitors. There are numerous trials, which have evaluated the potential merits of GPIIb/IIIa inhibitors during PCI comparing to placebo. A recent meta-analysis evaluating 21 randomised controlled trials which included 23,941 patients comparing GPIIb/IIIa inhibitors and placebo inferred a reduced 7 day post-procedure MI incidence in the GPIIIa/IIb treated group (4.31% vs. 6.97%, OR 0.59 CI 0.46-0.75) (214).

The relative clinical efficacy of abciximab, tirofiban, and eptifibatide at currently recommended doses has been uncertain for patients undergoing PCI. One meta-analysis in 2001 compared relative efficacy of individual GPIIbIIa inhibitors analysing 8 RCTs with 14644 patients. Abciximab resulted in a significant reduction of post-procedure MI from 8.5% to 4.3% (OR 0.49; 95% CI 0.40 to 0.59); compared to tirofiban and eptifibatide both of which showed non-significant reduction in MI from 6.9% to 5.9% (OR 0.85; 95% CI 0.69 to 1.04) (215). In the TARGET RCT (216) which directly compared tirofiban and abciximab involving 4809 patients during PCI, Abciximab significantly reduced the peri-procedural MI compared to tirofiban – an absolute 1·5% reduction of early myocardial infarction (6·9% *vs.* 5·4%, p=0·041). It was also recognised in this trial that patients with ACS undergoing PCI had the greatest benefit from abciximab.

Anti-thrombotics

Unfractionated heparin, which was the predominant anti-thrombotic treatment when PTCA was the only intervention, continues to be used in most PCI procedures. The other anti-thrombotics that have been evaluated in recent years include low molecular weight heparin and the direct thrombin inhibitors Bivalirudin and Fondaparinux. However, there have been

no randomised trials showing reduction in peri-procedural MI in elective PCI with any of the above anti-thrombotics.

Distal protection devices

Two types of embolic protection devices are in current usage, filters and aspiration (thrombectomy) catheters. Distal protection using filters appear to be of benefit in saphenous venous graft interventions but are not routinely used in PCI of native coronary arteries. In the SAFER trial (217) use of the embolic protection device in SVG interventions, with or without the concomitant administration of a glycoprotein IIb/IIIa inhibitor, significantly reduced the incidence of myocardial infarction (8.6% versus 14.7 %) and the 'no-reflow' phenomenon (3% versus 9%).

Direct Stenting

Direct stenting of the coronary lesion without pre-dilatation was found to reduce post-procedural Troponin I levels over 24 hours in a randomised controlled trial involving 311 patients by Nageh *et al.* (218). In a recent study by Cuisset *et al.* (219), direct stenting in stable-angina patients was associated with reduced microvascular dysfunction induced by PCI as compared with conventional stenting. It appears that further studies are required to establish the usefulness of direct stenting in reduction of peri-procedural myocardial injury. One meta-analysis comparing outcomes of direct stenting with conventional stenting found no benefit in MACE with non-significant trend towards reduction in early MI and death post-PCI (220). Prospective trials will be needed, as the use of direct stenting may select less complex lesions in which less PMI might be expected.

Strategies for protecting the myocardium against PMI (Cardioprotection)

Much of the existing medical therapy administered during PCI is to maintain the patency of the coronary artery and comprises primarily of anti-platelet and anti-thrombotic therapy. In cases of coronary no-reflow, intracoronary adenosine, calcium channel blockers or nitrates may be administered with the intention to improve myocardial reperfusion and maintain microvascular perfusion. However, an attractive alternative strategy may involve rendering

the myocardium resistant to the detrimental effects of PMI by pharmacological or novel interventions.

Statins

In addition to its lipid-lowering effect, some of the beneficial effects elicited by this class of drugs may be attributed to a variety of non-lipid lowering pleiotropic effects, including improved endothelial function, reduced oxidative stress, less platelet adhesion, and increased atherosclerotic plaque stability (221). Nitric oxide has been implicated as a crucial signalling molecule in cardioprotection and is involved in the other pleiotropic effects of statin therapy (221).

Although previous observational prospective and retrospective studies had shown reduction of peri-procedural myocardial damage in patients on pre-procedural statin (222, 223), it was in 2004 when ARMYDA trial (224) established that a significant reduction in post-procedure cardiac enzymes occurred in patients randomised to 40mg Atorvastatin for 7 days prior to the elective PCI compared to statin-naive patients. The recent NAPLES II trial (2009)(225) randomised 668 patients undergoing elective PCI studied the effect of a single 80mg loading dose of Atorvastatin within 24 hours before stent deployment compared to a control statin naive group. A significant reduction in peri-procedural MI was shown in the Atorvastatin group (9.5% vs. 15.8% OR: 0.56; 95% confidence interval: 0.35 to 0.89; p = 0.014). ARMYDA-ACS trial (226) which involved non-ST elevation myocardial infarction patients showed that the post-procedural elevation of CK-MB and Troponin-I was significantly lower in patients randomised to a loading dose of 80mg atorvastatin 12 hours before PCI and further 40mg pre-PCI compared to control group (7% vs. 27%, p = 0.001 and 41% vs. 58%, p = 0.039, respectively). Yun *et al.* (227), in their recent study on ACS patients, showed reduction in PMI in patients with single high-dose Rosuvastatin loading when compared to control group (11.4% versus 5.8%, p=0.035).

The ARMYDA-RECAPTURE trial (228) studied Atorvastatin reload in patients on chronic statin therapy undergoing percutaneous coronary intervention. They randomised 383 patients with both stable angina (53%) and non-ST-segment elevation acute coronary syndromes (47%) who were on previous statin therapy to have 80 mg loading of atorvastatin 12 hours before PCI and further dose of 40mg atorvastatin pre PCI versus a non

loading control group. This study inferred a lower incidence of post-procedural CK-MB and Troponin-I elevation > ULN in the atorvastatin arm (13% vs. 24%, p = 0.017, and 37% vs. 49%, p = 0.021, respectively). Of note, the stable angina patients who had PCI did not benefit from atorvastatin reload. A meta-analysis in 2007 by Merla *et al.*(229) evaluating 9 trials with 4751 patients, which assessed the impact of statin pre-treatment on peri-procedural myonecrosis, found 9% incidence in the statin-treated group compared 17.5% in the control group (OR 0.45, 95% CI: 0.33 to 0.62, p <0.01).

Beta Blockers

Intra-coronary propranolol at a dose of 15 µg/kg administered across the stenosis before the balloon dilation was shown to reduce peri-procedural incidence of CK-MB and Troponin T elevation compared to control patients in 2003 by Wang *et al.* (230)*.* When intracoronary propranolol administered with the GPIIbIIIa inhibitor eptifibatide, the post procedural CK-MB was significantly reduced compared to placebo, (231) highlighting an additive benefit of a beta blocker with a GPIIbIIIa inhibitor. Post-PCI CK-MB elevation > ULN occurred significantly more frequently in the placebo (21.5%) than in the propranolol (12.5%) group (RR 0.42; 95% CI 0.09, 0.63; P = 0.016) (231). It has been suggested that reduction in myocardial oxygen consumption may have accounted for the observed beneficial effect of propranolol. Another mechanism of benefit that has been suggested is an increase in the endocardial/epicardial ratio of tissue perfusion in the ischaemic area (232).

Adenosine

Adenosine is a naturally occurring nucleoside with a half-life in blood of less than 10 seconds. Adenosine, administered via the intravenous or intracoronary route, produces a hyperaemic effect that is commonly used in the measurement of coronary flow reserve and fractional flow reserve during PCI (233). Intra-coronary adenosine infusion, which pharmacologically mimics preconditioning, has been shown to significantly decrease peri-procedural cardiac enzyme rise in a small randomised study by Desmet *et al.* (234) in 2002, involving 28 patients. This study, however, involved infusion of adenosine over 10 minutes followed by 90 seconds of balloon inflation. A more recent randomised placebo controlled trial involving 62 patients undergoing non urgent PCI, showed reduction in peri-procedural biomarker

elevation in patients randomised to receive a bolus of intracoronary adenosine 50μg before 'wiring' as compared to placebo group (adjusted OR = 0.19, 95% CI:0.05–0.75, P =0.017) (235). Larger studies are required to establish adenosine's benefit and feasibility for routine adoption.

Trimetazidine

Trimetazidine is a piperazine derivative anti-anginal drug with a vasodilatory effect on coronary arteries and has been extensively studied due to its additional cardioprotective preconditioning properties. The mechanism of cardioprotection by trimetazidine appears to be modulation of mitochondrial homeostasis downstream of the preconditioning pathway (236). One placebo controlled randomised controlled trial involving 266 patients studied a loading dose of 60mg trimetazidine 30 min before re-canalisation; This study showed significant reduction in post-procedural Troponin I levels in the Trimetazidine group at all time points and also a significant reduction in the total Troponin I area under the curve (237).

Other Drugs of Promise

Cyclosporin-A and Erythropoietin are currently under investigation for usefulness in preventing peri-procedural myocardial injury. The cardioprotective effect of Erythropoetin appears to be due to activation of pro-survival kinases in the RISK (Reperfusion Injury Signalling Kinase) pathway (238) and that of Cyclosporin-A due to direct attenuation of mitochondrial permeability transition pore opening (mPTP) and preservation of mitochondrial function.(239) Cyclosporine's role in reducing reperfusion injury in primary PCI setting was shown by Ovize *et al.* (240) but its role in reducing PMI in elective PCI settings still needs to be established. Erythropoietin has been evaluated in the settings of acute MI patients undergoing primary PCI and the results have been disappointing and in fact showing higher adverse event rates (241, 242).

Non pharmacological interventions

Ischaemic and Remote Ischaemic Preconditioning

Ischaemic preconditioning and remote ischaemic preconditioning in the context of peri-procedural myocardial injury and peri-operative myocardial injury will be discussed at the end of this section in detail.

1.10.2 Therapeutic strategies to protect from peri-operative myocardial Injury during CABG

The myocardial injury sustained during CABG is dependent on mainly the type of cardiac surgery, the duration of surgery in terms of cross clamp time and cardio pulmonary bypass time, concomitant valve surgery, the type of electro-mechanical arrest strategy used for myocardial preservation (cardioplegia/cross clamp fibrillation/ off pump surgery) and substrate enhancement of the cardioplegic solution. Along with these, direct handling of the heart and injury due to cardiotomy through myocardium that is performed in valvular surgeries are other sources of myocardial enzyme release.

Consequently the focus in improving the outcomes during CABG has always been, to maintain low ischaemic time, improving the cardioplegic substrate enhancement, and maintenance of hypothermia. These strategies and their evolvement over the years have been discussed in the earlier section. There are, however, other cardioprotective interventions, which can modify ischaemia-reperfusion injury and these will be discussed here. Broadly these can be summarised as

1. Preconditioning
2. Antioxidants
3. Calcium channel blockers
4. Phospholipase A2 inhibitors
5. Na^+-H^+ echanger inhibitors
6. Protein phosphatise inhibitors
7. Phosphodiesterase inhibitor – pentoxifylline
8. 5 HT receptor antagonists.

Preconditioning

Ischaemic preconditioning, remote ischaemic preconditioning and anaesthetic preconditioning in the settings of CABG would be discussed in detail at the end of this section.

Antioxidants

Reactive oxygen species (ROS) in the form of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), reactive nitrites and nitrates are well known to be produced during ischaemia reperfusion injury (243). By their nature, ROS can attack virtually all cellular targets nonspecifically. In addition to altering membrane integrity and permeability, ROS also denature proteins, causing a loss in normal enzyme activity (244). In addition, they interfere with the sarcoplasmic reticulum calcium transport and potentiate inflammatory responses, by acting as chemotactic agents (244). Therefore exogenous antioxidant administration has been studied in ischaemia reperfusion injury settings. N- acetyl cysteine (NAC) has been shown in clinical CABG settings, to reduce ROS levels in coronary sinus blood but studies looking at post-operative myocardial enzymes are not promising (245, 246). Similarly, Allopurinol has been shown to reduce inotropic score and hospital stay, when used preoperatively, in patients undergoing valvular heart surgery, but without much effect on myocardial enzyme levels (247). In the laboratory settings, superoxide dismutase (SOD), Coenzyme Q10, oxypurinol, N-2 mercaptopropionyl glycine, melatonin and desferrioxamine have all been shown to be beneficial in ischaemic reperfused hearts (248-251).

Calcium Channel Blockers

Calcium channel blockers are known to protect myocardium in ischaemia reperfusion settings through various mechanisms, which include coronary vasodilatation, energy sparing effect resulting in lower ATP depletion, slower loss of adenosine precursors, protection of sarcolemma, and retardation of early rise in cytosolic Ca^{2++}(252). Diltiazem infusion peri-operatively has been shown to reduce postoperative myocardial enzyme release in one study(253) and intravenous nifedipine in another study on CABG patients has been shown to decrease ischaemic burden (254).

Phospholipase A2 Inhibitors

Phospholipase A2 inhibitors, which aid in protecting cell membrane integrity, are known to render protection against ischaemia-reperfusion damage. Manoalide, Mepacrine, chlorpromazine and MR-256 are known to be beneficial in myocardial ischaemia reperfusion settings in animal models; their exact mechanism of protection is still largely unknown, with no human clinical studies yet (255-257).

Na⁺-H⁺ Exchanger inhibitors

As discussed previously Na⁺-H⁺ Exchanger activation under ischaemic condition with low pH, leads to further Na⁺ retention, which leads to Na-Ca exchanger activation leading to calcium entry to cell, which leads to intracellular damage. Therefore Na-H inhibitors are researched for their cardioprotective effects. Amiloride and Cariporide are shown in animal heart models to reduce infarct size, in myocardial ischaemia reperfusion models (258). Cariporide is shown to cardioprotect in clinical setting of CABG with reduction in postoperative MI and mortality in the CABG subgroup of the GUARDIAN study (259). However, recently published EXPEDITION trial showed slightly higher mortality in Cariporide treated patients, although incidence of perioperative MI was significantly reduced in this group (260).

Protein phosphatase inhibitors, phosphodiesterase inhibitor- pentoxifylline and 5-HT receptor antagonists are the other agents studied in the settings of ischaemia reperfusion injury, however it is yet to be shown if they are cardioprotective in the clinical settings (252).

1.11 Ischaemic Preconditioning in Myocardial Revascularisation

Myocardial Ischaemic preconditioning, as described in previous section is a powerful endogenous cardioprotective phenomenon protecting heart from lethal ischaemic insult, when pre-exposed to sub lethal episodes of ischaemia and reperfusion. As myocardial revascularisation is a typical example of predictable settings of ischaemia reperfusion injury, preconditioning is a very attractive concept in this setting to prevent myocardial injury. Consequently, this strategy has been evaluated in both PCI settings and CABG settings. Laskey in 1999 (261), in a group of patients with various unstable ischaemic syndromes undergoing PTCA, showed that a preconditioning protocol of 90 second balloon inflation of

the target artery followed by 5 minute reperfusion, a cycle repeated twice, reduced CK enzyme elevation post procedure. However, this study was not strictly evaluating 'preconditioning', as patients in this study included acute MI patients and unstable angina patients. In MI patients, the protocol applied cannot be called IPC, rather it is IPostC. During PTCA, multiple balloon inflations of each at least 60 seconds were routine, and therefore 90 seconds inflation as a preconditioning stimulus, was very much plausible. However, with the current PCI strategies with stent usage, balloon inflation rarely exceeds 30 seconds and multiple balloon inflations are not common. Consequently, a preconditioning stimulus of 2 cycles of 90 second balloon inflations with 5 minutes of reperfusion in between those inflations is not very attractive for most cardiologists, who probably view this as very invasive. In CABG settings, there are many studies, which have evaluated the effect of IPC. Our research group in 1993 was the first to demonstrate the beneficial effect of IPC in CABG settings. IPC with 2 cycles of 3 minute aortic cross clamping separated by 2 minutes of reperfusion, before the more prolonged intermittent cross-clamp fibrillation in these patients resulted in higher ATP levels in myocardial biopsies (262). In another study, our group showed reduction in myocardial injury as indicated by a significant reduction in Troponin –T release in similar CABG settings (263). Since then, there has been multitude of studies showing cardioprotective benefits in terms of cardiac enzyme release, improved LV function and lower ionotropic scores in patients undergoing IPC in both cardioplegia settings and in cross clamp fibrillation settings. These studies are reviewed in detail by Venugopal *et al.* (264). Very few studies have refuted the cardioprotective effect of IPC in CABG settings and it appeared that these studies had either different IPC protocols or different cardioplegia delivery method (265-267). In spite of these evidences, surgeons do not routinely adopt IPC as this intervention could prolong their surgery and due to lack of clarity regarding accurate preconditioning protocols.

1.12 Remote Ischaemic Preconditioning in Myocardial Revascularisation

Certainly being less invasive, remote ischaemic preconditioning (RIPC) has become an attractive strategy for reduction of revascularisation related myocardial injury. As the concept is relatively new, there are many ongoing studies evaluating the effect of RIPC in

various clinical cardiac settings. In the elective PCI settings, Hoole *et al.* (268) have shown that remote preconditioning by 3 cycles of 5 minute forearm ischaemia followed by 5 minutes of reperfusion reduced post procedure cardiac Troponins at 24 hours, as well as 6 months cardiac and cerebral events rate. A previous smaller study by Ilidromitis *et al.* (269) had failed to demonstrate any improvement in cardiac enzymes in single vessel disease patients undergoing elective PCI. As RIPC typically needs to be applied before the onset of sustained ischaemia, it cannot be applied in the settings of acute MI as one cannot predict the onset. Consequently remote post-conditioning strategies are used in these circumstances. This applies during thrombolytic therapy in acute MI as well. The other setting where RIPC could be possibly of benefit is in the setting of urgent in-hospital PCI other than ST elevation MI presentations. These settings include patients presenting with Non-ST elevation acute coronary syndromes (NSTEACS). In these patients, there are unstable plaques in coronary arteries; however the vessel is still patent. So, the predominant injury sustained during PCI procedures is peri-procedural myocardial injury (PMI) to which RIPC might be of benefit similar to the benefit in elective PCI settings. The other settings where RIPC could be of potential benefit include rotablation for chronic total occlusions, and in settings of trans-catheter aortic valve implantation (TAVI). Both these settings are known to release large embolic particles, and consequently myocardial injury could be expected. In CABG settings, our group was the first to show that RIPC with 3 cycles of 5 minutes fore-arm ischaemia and 5 minutes of reperfusion using a blood pressure cuff, could reduce postoperative Troponins over 72 hours by 43% (79). This study included CABG patients receiving blood cardioplegic arrest or cross clamp fibrillation as a surgical cardioprotection strategy. In a separate study, involving only patients receiving blood cardioplegia, we have shown that similar trend persisted with 42% reduction in postoperative mean Troponin levels (270). Diabetic patients were excluded in this study. A recent study by Thielman *et al.* (271) has shown that in patients receiving crystalloid cardioplegia during CABG procedures for 3 vessel disease, RIPC reduced postoperative Troponins over 72 hours by 44.5%. The two latter studies described included a non-diabetic population only. However, two recent studies have failed to demonstrate any clinical or biochemical superiority of RIPC in the settings of elective CABG. These include a large double blind randomised study involving 162 patients undergoing elective CABG with standard blood –cardioplegia by Bonser *et al.* (272) and another smaller

study involving 54 patients undergoing elective CABG with either cardioplegia or cross – clamp fibrillation as myocardial preservation strategy by Kunst *et al.* (273).

1.13 Anaesthetic Preconditioning

Pharmacological mimics of ischaemic preconditioning have always interested researchers. Interestingly, few volatile anaesthetics, which are routinely used in major surgical procedures have been identified to possess inherent ability to induce preconditioning in myocardium. Kersten *et al.* (274) in 1997, first showed that isoflurane mimics ischaemic preconditioning *via* activation of K_{ATP} channels in canine hearts. This finding also triggered more research into the mechanisms involved in anaesthetic preconditioning. Subsequently, the involvement of protein kinase C, mitogen-activated protein kinases, adenosine type 1 (A_1) receptors, reactive oxygen species, and endothelial nitric oxide synthase have been shown to mediate volatile aneasthetics mediated preconditioning (275-280). It was initially thought that IPC and anaesthetic preconditioning shared the same signal pathway. However, in 2004, Sergeev *et al.* (281) demonstrated that anaesthetic induced preconditioning was associated with a more homogenous and predictable cardioprotective phenotype at a transcriptional level compared with IPC. Using a proteomic approach, it was shown that volatile anaesthetics induce long lasting changes in the expression profile of 106 proteins, which are related to their cardioprotective effect (282). Recently, involvement of pro-survival kinases such as PI3/Akt(283) and the transcription factor HIF-1(284) has also been shown with volatile anaesthetic preconditioning. Isoflurane, enflurane, halothane and recently sevoflurane are the volatile anesthetic agents mostly studied so far.

It is important to note that β-blockers may adversely affect anaesthetic preconditioning. Several studies have shown that anaesthetic mediated cardioprotection is abolished by propranolol and esmolol during anaesthetic induced preconditioning (285-287). This is particularly relevant in patients undergoing cardiac surgeries as most of the patients are on β-blockers as anti-anginal medication and/or as cardiac failure medication. Another important context where anesthetic preconditioning is shown to have less effect is in patients with diabetes and in the context of hyperglycemia (288). Diabetic hearts are less susceptible to anaesthetic preconditioning, possibly due to sharing similar pathways of

ischaemic preconditioning. Diabetes and preconditioning will be discussed in slightly more detail in the next section.

1.14 Myocardial Revascularisation in Diabetic Patients

The incidence of diabetes mellitus (DM) - a metabolic disease is increasing in an alarming rate throughout the world. Globally, the estimated diabetes prevalence for 2010 is 285 million and is expected to affect 438 million people by 2030 (289). In the UK, there are 2.6 million people who have been diagnosed with diabetes in the UK (2009) which is 4.1 % average prevalence and is estimated that 4 million people would be diabetic by 2025 (290). 90% of the people who have diabetes are Type 2 diabetic (291). The remarkable rate at which this particular disease has progressed confirms that diabetes is one of the biggest health challenges facing the UK today. Both Type 1 and Type 2 diabetic patients are at risk of cardiovascular complications. In the UKPDS Study (the largest prospective study of diabetes patients), macro-vascular disease (coronary artery disease, stroke and peripheral vascular disease) occurred in 20% of Type 2 DM, and macro-vascular disease was responsible for 59% of death in these patients (292). The impact of coronary artery disease in these patients has immense implications with increased MI incidence compared to non-diabetics. The Framingham heart study estimated that ischaemic heart disease accounts for more than 50% of death in diabetic patients (293). Type 2 diabetics without a prior infarction are at the same risk for MI and coronary mortality as non diabetics with a prior MI (294). It is now well known that diabetic patients also have worse prognosis after an acute myocardial infarct compared to non diabetics in terms of mortality, re-infarction rates and heart failure rates (295-297). Consequently, both primary and secondary care of a diabetic population who are at risk, and who present with ischaemic heart disease assumes a significant place in health care provision. Diabetic patients also have a higher incidence of multi-vessel disease compared to non-diabetic patients. In the TAMI trial 66% of the diabetic patients had multi-vessel disease (295).

The extent of coronary disease and its diffuse nature in a diabetic population poses unique challenges during revascularisation procedures. The prognosis after cardiac

revascularisation procedures is also worse in a diabetic population compared to non-diabetics. In patients undergoing PCI, diabetics have higher re-stenosis rates and lower event free survival compared to non diabetics (298, 299). This trend also applies to surgical revascularisation procedures. In patients undergoing CABG, both short term (30 days) and long term mortality (5yr and 10 yr) are higher among a diabetic population compared to non-diabetics (300, 301). It appears, therefore, that diabetic patients being more vulnerable to peri-operative risks should be better cardioprotected during cardiac surgeries.

1.14.1 Ischaemia Reperfusion Injury in Diabetic Heart

The diabetic heart response to ischaemic injury has been a matter of debate over many years. There exists controversy, as to whether the diabetic heart is more or less sensitive to ischaemic injury. Also, type 1 diabetic heart experiments and type 2 diabetic models have yielded different results, and this has further compounded the generalisation of ischaemic response in diabetic hearts. Among the animal models available, those developed in rodents have been studied most thoroughly, for reasons such as short generation time, inherited hyperglycaemia and/or obesity in certain strains, and economic considerations (302). Type 1 diabetes in rats is induced my injection of either Streptozocin or Alloxan, which are toxic glucose analogues, that preferentially accumulate in pancreatic beta cells via the GLUT2 glucose transporter. They are lethal to these cells and hence are diabetogenic. Type 2 diabetic rats are inbred rats and these spontaneously diabetic rodent models include OLETF rats, Goto Kakizaki (GK) rats, db/db mice, Zucker diabetic Fatty (ZDF) rats, and ob/ob mice. While the GK rats appear to be a suitable model for non-obese diabetes, ZDF rats are generally applied to studies of diabetes with obesity and cardiovascular complications due to the dyslipidaemia background (302).

In type 1 diabetic canine models, some studies have shown an increased susceptibility to ischaemia in the myocardium resulting in larger infarct size and more severe rhythm disturbances (303, 304). However, few studies involving rat models have shown that type 1 diabetic myocardium is paradoxically more resistant to ischaemia, resulting in limitation of infarct size (305). Similar findings were also shown in a rabbit diabetes model by Hadour *et al.* (306). In a recent *in vivo* study, Galagudza *et al.* (307) have shown that alloxan induced

diabetic rats of more than 6 weeks duration, had significantly less infarct size compared to controls for 30 minute ischaemia followed by 60 minute reperfusion, and ischaemic preconditioning has no further protective effect on diabetic hearts. Compared to in-vivo studies, most of in-vitro models have been consistent in demonstrating more resistance in diabetic hearts for severe ischaemia (305, 308). The possible explanations for these discrepancies are reviewed by Feuvray *et al.* (309). and Paulson *et al.* (310). In summary, the sensitivity of the diabetic heart to ischaemic injury depends upon the experimental conditions. With only a few exceptions, most studies showing lesser sensitivity to ischaemic injury in diabetes model used animal models with short duration of diabetes, employed zero flow ischaemia and had glucose as the only metabolic substrate. Most of the studies with longer duration of diabetes, if fatty acids were present and/or a model of low-flow ischaemia were used; the diabetic heart was more sensitive to ischaemic injury than non-diabetic hearts. The degree of flow reduction during ischaemia is thought to play an important role in determining the ability of diabetic hearts to recover from a period of ischaemia and reperfusion. During zero-flow ischaemia, glycolysis and subsequent acidosis may be detrimental to the cell. In these settings, the altered glucose metabolism in diabetic heart with decreased glycolytic flux is believed to be beneficial, as it attenuates production of glycolytic products –lactate and H^+ load. In addition, the diabetic heart exhibits a decrease in the activity of Na^+/H^+ and Na^+/Ca^{2+} exchangers, and in conjunction with lower glycolytic rates during ischaemia this may result in reduced accumulations of Na^+, and consequently during reperfusion, there is lesser gradient for reverse mode of Na^+/Ca^{2+}exchanger. This results in reduced Ca^{2+} influx limiting the injury in the diabetic heart. In contrast, during low flow ischaemia, the degree of intracellular lactate is less due to washout. Under these conditions, glycolysis may be beneficial, particularly to the diabetic heart that already has reduced rate of glycolysis. Many of the studies showing increased sensitivity to ischaemic injury had employed low flow ischaemia in their models (311-314).

The duration of the diabetes induction has also been an important factor in determining the sensitivity of ischaemia in diabetic myocardium. Two stages of experimental diabetes mellitus are identified- acute (1-3 weeks) and chronic (6-9 weeks) (315). Diabetes induction for more than 6 weeks in rat models, have mostly yielded results showing

increased sensitivity to ischaemia. Tosaki *et al.* (316) clearly demonstrated that even in the zero-flow ischaemic conditions, the duration of a diabetic state influences the ischaemia reperfusion injury. In their isolated heart models, 2 week old diabetic hearts demonstrated reduced incidence of ventricular arrhythmias and improved contractile function, relative to age matched control hearts following 30 minute global ischaemia followed by reperfusion. This protection was lost in 4-week and 6-week diabetic hearts, and 8-week old diabetic hearts exhibited depressed recovery after global zero-flow ischaemia compared to the control rats.

In isolated heart models of type 1 diabetes, lesser susceptibility to reperfusion arrhythmias in the initial 2 weeks of diabetes induction has been shown and this protection appears to be lost with progressive diabetes (4,6 and 8 weeks) (316).

Lastly, presence of exogenous fatty acids in the perfusion medium does influence the ischaemia reperfusion injury in diabetic heart models. This is particularly relevant in diabetes hearts, as hyperlipidaemia and diabetes are commonly coexistent. In DM1 rat models perfused under diabetic substrate conditions (elevated glucose and fattyacids) diabetic hearts recovered less contractile function compared to non-diabetic hearts even in presence of zero flow ischaemia (317). The exact mechanisms of the detrimental effect of fatty acids on IR injury specifically to DM hearts are still unclear.

Most of the studies on diabetic rat models have been on type 1 DM. There are limited studies evaluating type 2 DM (inbred) rat models for the response to ischaemia and reperfusion. The results of few studies, which have evaluated type 2 DM rats, have been interesting.

In type 2 diabetic inbred rat models, both Zucker fatty rats and Goto Kakizaki rats were shown to be more resistant to ischaemic insult compared to non-diabetic rats, in a Langendorff perfused model for a 50 minute ischaemia followed by 120 minutes of reperfusion (318). These rats were studied when they were 16 weeks old. In another study by Anzawa et al (319), who evaluated ischaemic response in another inbred type 2 diabetic male Otsuka Long-Evans Tokushima Fatty (OLETF) rats at 16 and 32 weeks of age, it was shown that compared to control age matched non diabetic hearts, 32 weeks OLETF rats had exacerbated acidosis in their coronary effluent indicating greater propensity of ischaemic insult. This study, however did not find significant differences in acidosis of coronary

effluent, between 16 weeks OLETF rat hearts compared to their age matched non- diabetic rat hearts. In an *in vivo* study by Yue *et al.* (320), ZDF rats (12 to 14 weeks old) were shown to have bigger infarct size compared to Zucker lean rats (Non diabetes rats) for 30 minutes of LAD occlusion followed by 24 hours of reperfusion. Similar findings were reported in a prior *in vivo* IR model in db/db type 2 DM rats by Jones *et al* (321). Therefore, type 2 DM animal models have also revealed contradictory findings on the susceptibility to injury during ischaemia and reperfusion. It is a well-known phenomenon that type 2 diabetes is dominated by hyperinsulinaemia, hyperglycaemia, and dyslipidaemia, while Type 1 diabetes is characterised by insulinopenia, hyperglycaemia, and a largely unaffected lipid profile (318).

1.14.2 Preconditioning the Diabetic Myocardium

Although the susceptibility to ischaemia reperfusion injury in experimental and *in vivo* animal diabetic models has always been unequivocal, most studies evaluating whether ischaemic preconditioning would reduce IR injury in diabetic models have consistently shown inability in reducing IR injury. There was however, one study by Tatsumi T *et al.* (322) that observed reduction in IR injury when type 1 DM rats were preconditioned with 2 cycles of 5 min ischaemia and 10 min reperfusion before the rat hearts were subjected to a 30 min sustained ischaemia, in a isolated perfused model. These were Male Sprague-Dawley rats after 4 weeks of Streptozozin induced DM1. Ravingerova T *et al.* (323), in their Langendorff-perfused model of Streptozocin-induced DM1 rats, observed that rats in their early phase of diabetes (1 week) were much resistant to IR injury related ventricular arrhythmia, and preconditioning with 1 cycle of 5 min ischaemia followed by 10 min reperfusion had no further benefit in subsiding V arrhythmia. However, older DM1 rats (9 weeks) had increased V arrhythmia comparable to non-diabetic hearts, and preconditioning significantly reduced V arrhythmias in this older diabetic group. Interestingly, a recent *in vivo* experiment by Galagudza *et al.* (307), which used 6 week old Alloxan-induced DM1 male Wistar rats, showed very different results from those of Ravingerova T *et al*. (307). In this study, DM1 rats had significantly less infarct size than control rats for 30 min sustained ischaemia, and

also had significantly less V arrhythmia. IPC with one episode of 5 minute ischaemia and reperfusion in DM rats, did not have any effect on infarct size reduction, and had only subtle beneficial effect on V arrhythmia (307).

In type 2 diabetic inbred rat models, both Zucker fatty rats and Goto Kakizaki (GK) rats were shown to be more resistant to ischaemic insult compared to non-diabetic rats, and also preconditioning with 4 cycles of 2 min ischaemia and 3 min reperfusion had no beneficial effect in limiting infarct size in either of the diabetic rats (318). In our laboratory, we showed that in GK rats, a prolonged preconditioning protocol with 3 cycles of 5minute ischaemia and 10 minute reperfusion can reduce the infarct size, however, with lesser preconditioning stimulus, the protection is not seen (324). The control group in this experiment was non-diabetic Wistar rats. The diabetic GK hearts did show reduced infarct size to the index IR injury, compared to controls. It was also shown that this increased threshold was likely due to a defect in the PI3 Kinase- Akt pathway, resulting in lesser Akt phosphorylation in GK rats (324). This was the first study, which demonstrated that diabetic myocardium could be preconditioned, albeit with a higher threshold. The total Akt levels were the same in both diabetic rats and control rats, but it appears that phosporylation of Akt is deficient in diabetic rats. In a human atrial trabeculae model from our lab, Sivaraman *et al.* (325) showed that prolonged hypoxia preconditioning of 7 minutes followed by 16 minute reperfusion was cardioprotective in contractility recovery after sustained ischaemia in diabetic patients, whereas the standard protocol with 4 minute hypoxia could not caardioprotective. In contrast, control non-diabetic patients' trabeculae had a cardioprotective effect even with the standard preconditioning protocol. Basal levels of Akt phosphorylation were less in trabeculae from diabetic patients. In another GK rat model, from our lab, it was shown that levels of Phosphatase and Tensin homologue on chromosome 10 (PTEN), a recently discovered enzyme responsible for negatively regulating PI3 kinase pathway, is elevated in diabetic rat hearts (326). A recent study by Hassouna *et al.* (327) has implicated mitochondrial dysfunction to the impairment of IPC in diabetic myocardium, raising possibility of dysfunctional mitochondrial K_{ATP} channels.

In summary, it appears that the diabetic hearts from animal models in particular circumstances, are more resistant to ischaemic injury, and although further reduction of this IR injury is possible through preconditioning, it requires an increased preconditioning stimulus.

2. AIMS AND OBJECTIVES

In spite of significant advances in both surgical and percutaneous myocardial revascularisation procedures, significant peri-operative and peri-procedural myocardial injury still occurs, leading to worse outcomes after revascularisation procedures. Some groups of patients are at a higher risk of sustaining myocardial injury during revascularisation, and these subgroups would need better cardio-protection during these procedures so that the myocardium could resist and cope with the ischaemia-reperfusion injury sustained. As discussed in the previous chapter, patients with non-ST elevation acute coronary syndrome (NSTEACS) undergoing urgent in-hospital PCI are at an increased risk of peri-procedural myocardial injury. Similarly, in the setting of CABG, type 2 diabetic patients sustain increased peri-operative myocardial injury, and have increased morbidity and mortality.

We evaluated the cardio-protective intervention (Remote Ischaemic Preconditioning) in these two high risk subgroups of patients, who are known to be susceptible for increased myocardial injury during revascularisation procedures i.e. a) patients who have had NSTEACS undergoing urgent in-hospital PCI procedures, and b) Type 2 diabetic patients undergoing cardiac bypass surgery.

2.1 Overall Hypothesis

Remote ischaemic preconditioning reduces myocardial injury in the myocardial revascularisation settings of coronary angioplasty and coronary artery bypass graft surgery.

Hypothesis 1

Remote Ischaemic preconditioning reduces myocardial injury in non-ST elevation acute coronary syndrome (NSTEACS) patients, undergoing urgent in-patient coronary angioplasty.

Hypothesis 2

Remote Ischaemic preconditioning reduces myocardial injury in Type 2 diabetic patients, undergoing elective coronary artery bypass surgery.

2.2 Overall Aim

To study the effect of remote ischaemic preconditioning on the myocardial injury in NSTEACS patients undergoing coronary angioplasty, and to study the effect of remote ischaemic preconditioning on the myocardial injury in Type 2 diabetic patients undergoing elective coronary artery bypass surgery.

2.3 Objectives

a) To study the effect of remote ischaemic preconditioning on myocardial injury in consecutive patients admitted with NSTEACS (Unstable angina or Non-ST elevation myocardial infarction-NSTEMI), undergoing urgent in-hospital coronary angioplasty.

b) To study the effect of remote ischaemic preconditioning on myocardial injury in Non-ST elevation myocardial infarction (NSTEMI) subgroup of NSTEACS patients, undergoing urgent in-hospital coronary angioplasty.

c) To study the effect of remote ischaemic preconditioning on myocardial injury in consecutive Type 2 diabetic patients, undergoing elective coronary artery bypass graft surgery with or without aortic valve surgery, irrespective of surgical myocardial preservation technique.

d) To study the effect of remote ischaemic preconditioning on myocardial injury in Type 2 diabetic patients, undergoing elective coronary artery bypass graft surgery, with or without aortic valve replacement using cardioplegia as the technique of myocardial preservation.

e) To study the effect of remote ischaemic preconditioning on short term outcomes after elective coronary artery bypass surgery in Type 2 diabetic patients. The short-term

outcomes selected in this study are based on those measured in other clinical studies on IPC and RIPC:

1. Duration of ventilation support.

2. Duration of intensive care unit stay.

3. Inotrope score.

4. Acute kidney injury over first three postoperative days.

5. Incidence of cardiac arrhythmias.

3. REMOTE ISCHAEMIC PRECONDITIONING IN NON-ST ELEVATION ACUTE CORONARY SYNDROME (NSTEACS) PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTIONS.

Remote ischaemic preconditioning in the form of transient limb ischaemia is a simple, non-invasive intervention, which is easily applicable to clinical settings. As discussed previously, the protocol was first standardised by Kharbanda *et al.* (67) in normal human volunteers with transient upper limb ischaemia as remote preconditioning stimulus in a study demonstrating endothelial protection. The same group subsequently showed that this remote preconditioning effect has both early and delayed phases, both of which were dependent on the neuronal mechanism that could be blocked by administration of ganglion blocker trimetaphan (69). In a later study, they also showed that the protective endothelial effects through contralateral limb ischaemia can be reproduced by administering the protocol after the index limb ischaemic episode – remote ischaemic postconditioning, and that both RIPC and RIPostC were dependent on the opening of the mito-K_{ATP} channel (328).

The first clinical study utilising this remote preconditioning protocol was performed by Cheung *et al.* (329), who demonstrated reduced Troponin I in children undergoing corrective surgery for congenital cardiac defects. In the settings of coronary angioplasty, Hoole *et al.* demonstrated reduced Troponin elevation at 24 hours post procedure in patients undergoing elective PCI, who were randomised to remote preconditioning (268). Our current study hypothesises, that remote ischaemic preconditioning (RIPC) induced by transient limb ischaemia and reperfusion, would reduce myocardial injury in adult patients admitted with non-ST elevation acute coronary syndrome (NSTEMI and Unstable angina) undergoing inpatient coronary angioplasty.

3.1 METHODS

3.1.1 Overview

We conducted a two Centre, open label randomised controlled study of the effect of RIPC induced by transient limb ischaemia on myocardial injury during urgent inpatient coronary angioplasty, among consecutive patients admitted with non-ST elevation acute coronary syndromes.

3.1.2 Ethical Approval and Informed Consent

The protocol for this study was written in accordance with the International Conference on Harmonisation - Good Clinical Practice (ICH-GCP) guidance, and the study was subject to approval by the joint University College London (UCL)/University College London Hospitals (UCLH) committees for the ethics of human research. Approval for the study was sought on the standard application form initially provided by the NHS COREC (Central office for the Research Ethics Committees), and subsequently integrated with the National Research Ethics Service (NRES), affiliated to the National Patient Safety Agency (NPSA). In addition to the study protocol, the patient information sheet, consent form, and letter to the patient's general practitioner, were also approved by the ethical committee.

Once ethical approval was obtained, a separate application was made to the research and development (R&D) department UCLH, who also acted as sponsors of the study. Any amendments to the protocol were classed as major or minor, as per advice from the ethical committee. I made the amendments and submitted the documents, annotated with appropriate dates and version numbers for approval for each amendment. A separate site-specific information (SSI) form was submitted to Surrey Research Ethics Committee, Guildford for including a second recruitment centre, East Surrey Hospital.

Any serious adverse event or reaction during the course of the study had to be reported to the R&D department as per guidelines. No serious adverse events or reactions occurred during the course of this study.

Patients admitted with NSTEMI and unstable angina awaiting urgent inpatient coronary angiography, were approached after a decision for the coronary angiography was made. Informed consent was obtained using the current version of the patient information sheets, and consent forms, and patients' signatures obtained. A copy of the signed consent form and the patient information sheet were given to the patient, and the original forms filed in the hospital notes. An additional copy of the signed consent form was filed at the Hatter Cardiovascular Institute, for patients recruited at the Heart Hospital, and at East Surrey Hospital for patients recruited at Surrey and Sussex NHS Trust.

3.1.3 Patient Recruitment

Consecutive patients admitted with NSTEACS, between September 2008 and September 2010, were screened for recruitment into the study. At the East Surrey Hospital site, the patient recruitment for this study was started from February 2009.

3.1.4 Inclusion Criteria

- Age > 18 years.

Consecutive patients admitted with NSTEACS (NSTEMI and Unstable angina) undergoing in-hospital coronary angioplasty. NSTEMI is defined as Troponin positive chest pain among admitted patients. Unstable angina is defined as cardiac sounding chest pain, with ECG changes of myocardial ischaemia among admitted patients.

3.1.5 Exclusion criteria

- Renal failure (EGFR <35 ml/min/1.73m^2)

- Patients with Troponins levels, which are not stable and increasing, in bloods taken at least 6 hours apart before the coronary angiography.

- Peripheral vascular disease affecting upper limbs

- Cardiac arrest in the present hospital admission

- Patients on drugs Nicorandil or Glbenclamide.

3.1.6 Randomisation and Transient Limb Ischaemia Protocol

Patients were randomised to either the control group or the RIPC group, using electronic randomisation methods of random sequence generation for the two treatments. A simple randomisation method was used to allocate patients to receive either RIPC or a placebo. http://www.random.org/sequences/

RIPC was induced by transient limb ischaemia in patients randomised to receive the intervention. This was achieved by inflating a standard 9 inch blood pressure cuff to 200 mmHg on the upper arm for five minutes, for three cycles each separated by a reperfusion period of five minutes, during which time the cuff was kept deflated. A similar protocol was first used by Kharbanda *et al.* (67), to induce remote endothelial preconditioning in the contra-lateral limb in normal human volunteers.

The protocol was applied to the right arm whenever possible, but if a continuous intravenous fluid/drug infusion was already established in right arm, the RIPC protocol was applied on the left arm. The protocol was commenced on the day of the angiography procedure and was timed around an hour before the coronary angiography procedure. If the angiography was delayed more than three hours after RIPC, the patient was excluded from the study. Loukogeorgakis *et al.* (69), in their characterisation model of remote preconditioning in human volunteers, have shown that the endothelial protection conferred by RIPC disappears after 4 hours of preconditioning stimulus.

3.1.7 Percutaneous Intervention (PCI) Procedure

Twelve lead ECG traces were recorded before the procedure, and the findings were recorded in all recruited patients. The consultant performing the coronary angiography decided the choice of arterial access. Once the arterial sheath was inserted (femoral/radial/brachial), a blood sample from the arterial sheath was taken to determine the baseline (Zero hour) Troponin T and CKMB levels. The coronary angiography was done in all patients before a decision of proceeding to PCI was made. The interventional consultant responsible for the

coronary angiography determined if the patients were to proceed to PCI, based on the clinical and angiographic assessment.

If the patients did not proceed to PCI, they were excluded from taking further blood samples. The patients, who were proceeded to PCI were monitored throughout the procedure, and procedural details were clearly recorded. The interventional consultant performing the procedure determined the choice and sizes of catheters and stents. Detailed interventional events and parameters were recorded during the procedure. Any complications during the procedure were also recorded.

Post PCI procedure, all patients were transferred to either the recovery unit or coronary care unit, and any post procedure events until their discharge from the hospital were recorded. The interventional consultant determined if the patients were required to have GP3a2b inhibitor post procedure, and this was recorded.

3.1.8 Serum TroponinT and CK-MB Measurement

Blood samples were taken for cTnT and CK-MB levels at the time of arterial sheath insertion (0 hour), and at 6 hours, 12 hours, and 24 hours post PCI procedure. In both recruiting Centres, cTnT was measured quantitatively by a one-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010; Roche). The lower detection limit of this assay (99th percentile URL) is 0.01 μg/L, with a co-efficient of variation of 10%, and recommended diagnostic range of 0.03-0.09 μg/L indicating possible myocardial injury and a threshold of $\geq$ 0.1μg/L indicating myocardial injury suggestive of myocardial infarction.

CK-MB mass assay in both the recruitment centres were measured using Elecsys 2010 CK-MB STAT assay (Roche Diagnostics). The lower detection limit of this assay (99th percentile URL) is 4 μg/L and coefficient of variation < 10% with a recommended diagnostic level of > 4 μg/L indicating myocardial injury.

Enzyme level parameters were compared with respect to mean increase at individual time points, proportional increase from baseline levels, and area under the curve (AUC) of enzyme levels over 24 hours.

3.1.9 Statistical Analysis

Standard statistical methods were used for analysis. Histograms with normal plots were performed to check normal distribution in the two groups. Differences between the groups for continuous variables were tested using the analysis of variance (ANOVA) test, and for categorical variables using Chi-square test. The general linear multivariate model was used to test for multivariate analysis. Area under the Curve (AUC) analysis was performed using the trapezoid rule. Significance was interpreted at the 95% confidence interval, having corrected for any inequality of variance between the groups. The data analysis was based on intention to treat. Data were analysed using SPSS statistical software version 16.

3.1.9.1 Sample Size Calculation

In a recent large prospective study, the incidence of cTnT elevation >0.03μg/L after PCI procedures was reported to be 46% in patients with normal baseline cTnT levels, and 80% in patients who have already raised Troponins at baseline (330). As our patient cohort involved patients with unstable angina (normal baseline cTnT levels), and patients with NSTEMI (elevated baseline cTnT levels), we assumed an incidence of 60% for cTnT elevation >0.03μg/L, above the baseline level in our patient cohort undergoing PCI. Therefore, for a reduction of 20% absolute incidence from 60% to 40% we needed 95 patients in each group at 5% significance and 80% power.

3.2 RESULTS

A total of 293 patients were screened for inclusion into the study. 10 patients refused to take part in the study; 3 patients had baseline renal dysfunction (EGFR <35mls/min/1.73m^2); 3 patients were unable to consent due to dementia. Therefore, 277 patients were randomised. There were134 patients in control group, and 143 patients in RIPC group. All 277 patients

underwent coronary angiography. 158 patients did not proceed for PCI. 51 patients had normal coronary arteries, 58 patients were referred to coronary artery bypass surgery (CABG), and 39 patients were suitable for medical management only.

Among 119 patients who completed full study, 9 patients had their baseline Troponin levels (0 hour Troponin level) higher than the admission Troponin levels and therefore, excluded from further analysis. In total 110 patient's data were included in the final analysis. 58 patients were in control group, and 52 patients in RIPC group. 66 (60%) patients had NSTEMI with Troponin increase during admission, and 44 (40%) patients had unstable angina, with ECG changes of myocardial ischaemia at rest.
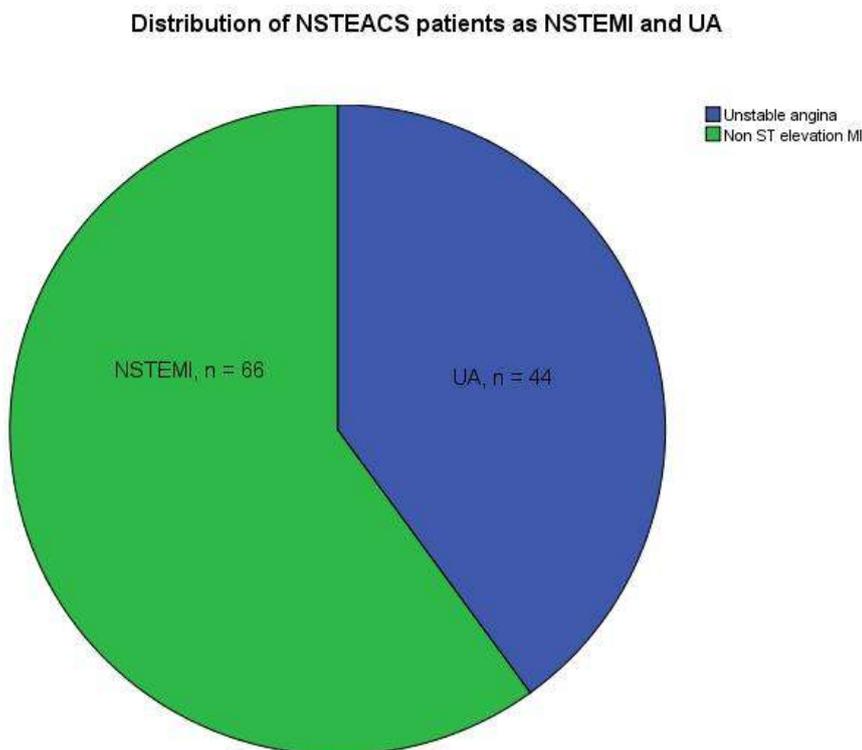
Fig 1.

Fig 2
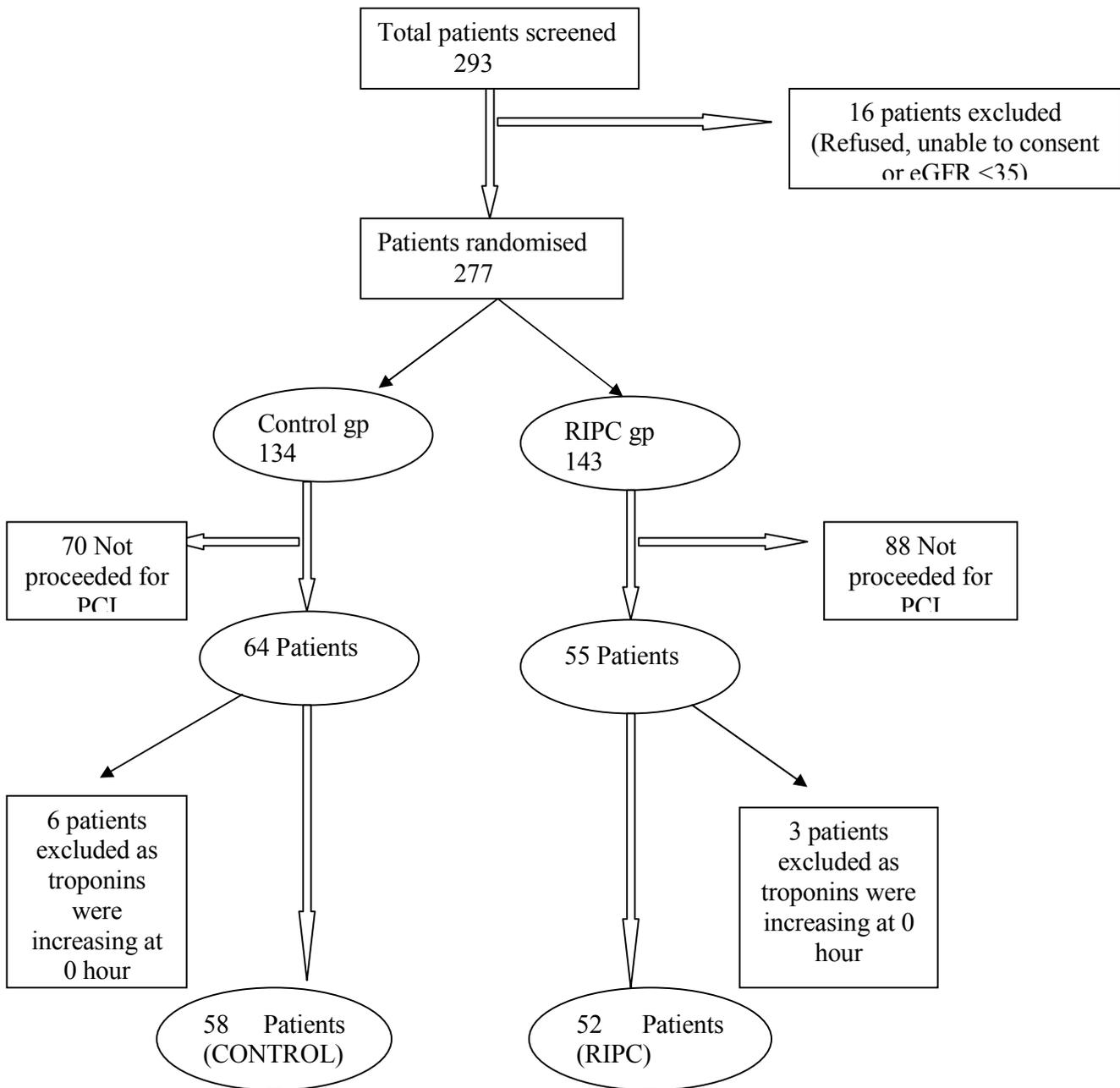
3.2.1 Baseline Characteristics- Patient Profile

The baseline characteristics of the entire cohort randomized and baseline characteristics of the patients who completed the study are depicted in the tables below separately I tables 3.01 and tables 3.1.

Table 3.01

Demographics	Control (n=134)	RIPC (n=143)
Age (years)	64.5	62.1
Male	81	96
Female	53	47
Previous Angina	68 (50.8%)	54 (37.7%)
Diabetes	40 (29.8%)	37 (25.8%)
Hypertension	63 (47%)	72 (50.3%)
Dyslipidaemia	48 (35.8%)	66 (46.1%)
Pulmonary disease	7 (5%)	12 (8%)
Mild Renal impairment	2 (1.4%)	2 (1.3%)
Previous MI	23 (17.1%)	27 (18.8%)
Current smoker	43 (32%)	54 (37.7%)
Last Chest pain (before PCI) (hours)	77	69
Time to Intervention from first presentation (days)	3.76	3.9
Baseline Creatinine level	76	81
Mean Troponin-T levels at admission	0.28 (SEM=0.06)	0.34 (SEM 0.06)
Mean Troponin-T levels at the time of PCI (0 hour level)	0.15 (SEM=0.04)	0.28 (SEM = 0.04))
Mean CKMB level at the time of PCI (0 hour level)	4.2 (SEM= 0.7)	5.32(SEM= 1.2)

Table 3.0.2

Drugs	Control	RIPC
Aspirin	120 (89.5%)	126 (88.1%)
Statin	90 (67.1%)	93 (65%)
Nitrate	14 (10.4%)	20 (13.9%)
Beta blocker	52 (38.8%)	49 (34.2%)
Ca^{2+} channel blocker	19 (14.1%)	27 (18.8%)
Sulphonylurea	12 (8.9%)	9 (6.2%)
ACE inhibitor	90 (67.1%)	86 (60.1%)

In patients who completed the study, there were no significant differences between the control and RIPC groups, with respect to most of the baseline characteristics. The control group, however, had more patients with previous history of angina (44% vs. 25%; p = 0.04). The other characteristics, which were tending towards statistically significant difference, were: a) Diabetes, and b) Smoking history. A higher proportion of patients in the control group had diabetes (20.7% vs. 7.7% p =0.06). RIPC group had more smokers compared to the control group (42% vs. 24% p =0.06).

The procedural delay from the time of admission to PCI was not different in the two groups (3.98 days vs. 3.78 days, p = 0.62). The procedural time delay from the time of the last chest pain was also not different between each group (79.1 hours vs. 67.3 hours, p = 0.23).

Table 3.1

Demographics	Control (n=58)	RIPC (n=52)
Age (years)	62.05	60.9
Male	45 (77.6%)	43 (82.7%)
Female	13 (22.4%)	9 (17.3%)
Previous Angina	26 (44.8%)	13 (25%)
Diabetes	12 (20.7%)	4 (7.7%)
Hypertension	25 (43.1%)	30 (57.7%)
Dyslipidaemia	20 (34.5%)	22 (42.3%)
Pulmonary disease	2 (3.4%)	4 (7.7%)
Mild Renal impairment	0 (0%)	1 (1.9%)
Previous MI	10 (17.2%)	11 (21.2%)
Current smoker	14 (24%)	22 (42%)
Last Chest pain (before PCI) (hours)	79.1	67.3

Time to Intervention from first presentation (days)	3.98	3.75
Baseline Creatinine level	82.21	82.92
Mean Troponin-T levels at admission	0.30 (SEM= .06)	0.35 (SEM=.07)
Mean Troponin-T levels at the time of PCI (0 hour level)	0.17 (SEM=0.04)	0.29 (SEM = 0.05)
Mean CKMB level at the time of PCI (0 hour level)	3.91 (SEM= 0.3)	5.86 (SEM= 1.4)

Table 3.1.1

Drugs	Control	RIPC
Aspirin	54 (93.1%)	50 (96.2%)
Statin	47 (81%)	44 (84.6%)
Nitrate	5 (8.6%)	7 (13.5%)
Beta blocker	22 (37.9%)	28 (53.8%)
Ca^{2+} channel blocker	9 (15.5%)	8 (15.4%)
Sulphonylurea	3 (5.2%)	1 (1.9%)
ACE inhibitor	25 (43.1%)	24 (46.2%)

3.2.2 Pre-procedure Drug Profile

There were no significant differences in the drug profile between both groups prior to the procedure. The drug profile of both groups is summarised in table 3.2.

3.2.3 Baseline Characteristics : Peri-procedural Variables

The peri-procedural variables in the two groups are summarised in table 3.3. All patients undergoing PCI had at least one stent deployed. Around 85% of patients in both groups had single vessel intervention. Most patients had drug eluting stents in both groups (90%). RIPC group had more patients receiving GPIIbIIIa inhibitors compared to control group, which was nearly statistically significant (13.7% vs. 3.5%, $p = 0.08$). There were no differences in

the number of balloon inflations, mean stent length or mean stent diameter between the two groups.

3.2 Peri-Procedure Variables

Variable	Control	RIPC
GPIIbIIIa inhibitors	2 (3.5%)	7 (13.7%)
No of vessels intervened (Mean)	1.16 (SEM= 0.048)	1.15 (SEM= 0.05)
No of balloon inflations (mean)	6.93 (SEM= 0.60)	6.45 (SEM= 0.59)
LAD intervention	25 (43.1%)	15(28.8%)
Multiple vessel intervention	9 (15.5%)	8 (15.4%)
Pre-dilation	55 (94.8%)	49 (94.6%)
Drug Eluting Stents	51 (87.9%)	47 (90.4%)
No of Stents (Mean)	1.57	1.56
Mean Stent length (mm)	33.06	30.96
Mean Stent diameter (mm)	3.04	3.22

3.2.4 Serum CTnT Release

Cardiac TnT levels were measured pre-procedurally (during access site puncture: 0 hour cTnT) and at 6 hours, 12 hours and 24 hours post procedure. The mean ($\pm$SD) cTnT levels at these time points are shown in table 3.3.

Table 3.3

Time (Hours)	Control	RIPC	Mean difference	Confidence interval	P Value
0	0.17 $\pm$0.36	0.29 $\pm$0.39	-0.113 $\pm$0.07	-0.25 - 0.03	0.12
6	0.24$\pm$0.46	0.41$\pm$0.61	-0.17 $\pm$0.10	-0.37 - 0.03	0.10
12	0.29$\pm$0.52	0.42$\pm$0.69	-0.13$\pm$0.11	-0.36 - 0.10	0.27
24	0.27$\pm$0.44	0.37$\pm$0.57	-0.09$\pm$0.10	-0.30 - 0.10	0.34

Values present mean $\pm$ SD (in µg/L)

Percentage rise in troponins from base line	6hrs	12hrs	24hrs
Control	41.1%	70.5%	58.8%
RIPC	41.3%	44.8%	27.5%

Fig 3 : Mean Troponins over 24 hours post PCI
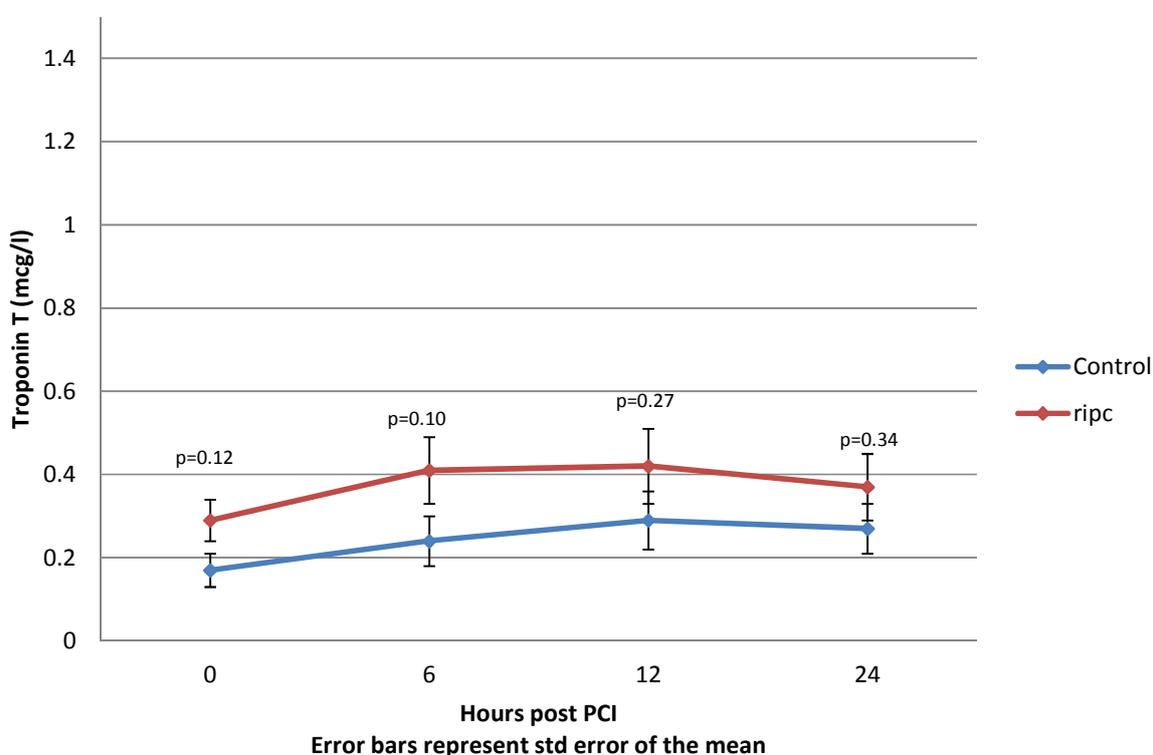
There was no statistical difference in the cTnT levels in the blood samples at any time intervals post procedure. The mean baseline Troponin levels (0 hour troponins) of the RIPC group was higher than that of control group, [Mean (SD) of RIPC group 0.29($\pm$0.39) µg/L vs. 0.17($\pm$0.36) µg/L in control group]. This baseline inequality leads to the appearance in the above graph, that higher Troponin level are released at individual time points in RIPC group. However, the Troponin area under the curve (AUC) over 24 hours adjusted to the baseline Troponin levels, showed no statistical difference between the two groups (Mean $\pm$SD of Troponin AUC in RIPC group 9.45$\pm$13.8 vs. 8.97$\pm$10.7 in the control group, p =0.83).

3.2.5 Periprocedural Myocardial Infarction incidence (PMI)

The incidence of PMI was calculated and compared between the two groups, and the results are summarised in the table 3.5. The new 'universal definition of myocardial infarction', defines peri-procedural myocardial infarction during PCI as an increase in cardiac enzymes more than 3 times ULN. In patients with already increased baseline Troponins, further increases in Troponin levels should be more than 20% of the baseline Troponin levels. An increase in cardiac enzyme level > 1 x ULN will still constitute periprocedural myocardial injury, however 3 x ULN increase will constitute periprocedural myocardial infarction.

In our cohort of patients with NSTEACS, 55% of the patients undergoing in-hospital PCI had PMI according to the new universal definition of MI. There was, however, no difference in the incidence of PMI between the two groups for occurrence of PMI. These results are summarised below.

Table 3.3.1

PMI definition	Control	RIPC	P value
>0.03µg/L increase in the cTnT level from baseline (0 hour) level	31 (53.4%)	29 (55.8%)	0.85
>20% increase in cTnT level from baseline (0 hour) level	34 (58.6%)	33 (63.5%)	0.69
>0.03µg/L increase and >20% increase in cTnT level from baseline (0 hour) level	30 (51.7%)	26 (50%)	1.0
CKMB mass increase >12µg/L (>3x ULN) from baseline level	5 (8.6%)	8 (15.4%)	0.37
Troponin increase > 1 x ULN (>0.01 µg/L) from baseline level	34 (58.6%)	33 (63.5%)	0.69
CKMB mass increase > 1 x ULN (>4.9 µg/L) from baseline level	18 (31%)	18 (34.6%)	0.83

3.3 Sub-group Analysis of Results in NSTEMI Cohort of Patients.

Our patient cohort consisted of both NSTEMI and Unstable angina patients needing urgent in-patient PCI. 60% of this cohort (66 patients) had NSTEMI, and 40% constituted UA. We sub-analysed the NSTEMI cohort for the PMI incidence applying the universal definition of

MI and compared between the two groups. The baseline characteristics and the results will be discussed below.

3.3.1 Baseline Patient Characteristics (NSTEMI COHORT)

The baseline characteristics in both groups are well matched, with respect to most of the variables. However, the control group had significantly more patients with pre-existing angina (35.5% vs. 8.6%, p = 0.01). In addition, the control group had a higher number of diabetic patients, which nearly reached statistical significant (25.8% vs. 8.6%, p = 0.10).

Table 3.4

Demographics	Control (n=31)	RIPC (n=35)
Age (years)	61.6	58.2
Male	25 (80.6%)	29 (82.9%)
Female	6 (19.4%)	6 (17.1%)
Previous Angina	11 (35.5%)	3 (8.6%)
Diabetes	8 (25.8%)	3 (8.6%)
Hypertension	16 (51.6%)	18 (51.4%)
Dyslipidaemia	11 (35.5%)	12 (34.3%)
Pulmonary disease	0 (0%)	2 (5.7%)
Previous MI	5 (16.1%)	6 (17.1%)
Current smoker	11 (35.5%)	18 (51.4%)
Last Chest pain (before PCI) (hours)	72.8	65.2
Time to Intervention from first presentation (days)	3.7	3.5
Baseline Creatinine level	80.1	80.3
Mean Troponin-T levels at admission	0.56	0.51
Mean Troponin-T levels at the time of PCI (0 hour level)	0.32	0.42
Mean CKMB level at the time of PCI (0 hour level)	4.5	7.2

3.3.2 Pre-procedure Drug Profile

There were no significant differences in the drug profile between both groups prior to the procedure. The drug profile of both groups has been summarised in table 3.4.

Table 3.4.1

Drugs	Control (n=31)	RIPC (n=35)
Aspirin	30 (96.8%)	35 (100%)
Statin	26 9(83.9%)	31 (88.6%)
Nitrate	1 (3.2%)	4 (11.4%)
Beta blocker	13 (41.9%)	20 (57.1%)
Ca^{2+} channel blocker	7 (22.6%)	4 (11.4%)
Sulphonylurea	1 (3.2%)	0 (0%)
ACE inhibitor	15 (48.4%)	16 (45.7%)

3.3.3 Baseline Characteristics: Peri-procedural Variables

The peri-procedural variables in the two groups are summarised in table 3.5. All patients undergoing PCI had at least one stent deployed. Around 85% of patients in both groups had single vessel intervention. There were no differences in the number of balloon inflations, mean stent length, or mean stent diameter between the two groups. The peri-procedural variables and their comparison between the two groups are listed in the table 3.5.

3.5 Peri-Procedure Variables

Table 3.5

Variable	Control (n=31)	RIPC (n=35)	P value
GPIIbIIIa inhibitors	2 (6.5%)	5 (14.3%)	0.43
No of vessels intervened (Mean)	1.16	1.17	0.91
No of balloon inflations (mean)	6.58	6.86	0.79
LAD intervention	12 (38.7%)	7 (20%)	0.11
Multiple vessel intervention	5 (16.1%)	6 (17.1%)	1
Pre-dilation	29 (93.5%)	33 (94.3%)	1
Drug Eluting Stents	27 (87.1%)	33 (94.3%)	0.40
No of Stents (Mean)	1.39	1.60	0.25
Mean Stent length (mm)	29.3	32.5	0.45
Mn Stent diameter (mm)	2.99	2.92	0.52

3.3.4 Serum Troponin T Release

Troponin T levels were measured pre-procedurally (during access site puncture: 0 hour cTnT), and at 6 hours, 12 hours, and 24 hours post procedure. The mean ($\pm$SD) cTnT levels at these time points are shown in table 3.6.

Table 3.6

Time (Hours)	Control	RIPC	Mean difference	Confidence interval	P Value
0	0.32 $\pm$0.46	0.42 $\pm$0.42	-0.10 $\pm$0.10	-0.32 - 0.11	0.34
6	0.41$\pm$0.59	0.59$\pm$0.67	-0.18 $\pm$0.15	-0.50 - 0.11	0.24
12	0.44$\pm$0.61	0.61$\pm$0.78	-0.16$\pm$0.17	-0.51 - 0.18	0.34
24	0.36$\pm$0.49	0.51$\pm$0.63	-0.15$\pm$0.14	-0.43 - 0.13	0.29

Values present mean $\pm$ SD (in µg/L)

Fig 4 : Mean Troponins over 24 hrs post PCI

Error bars represent std error of the mean
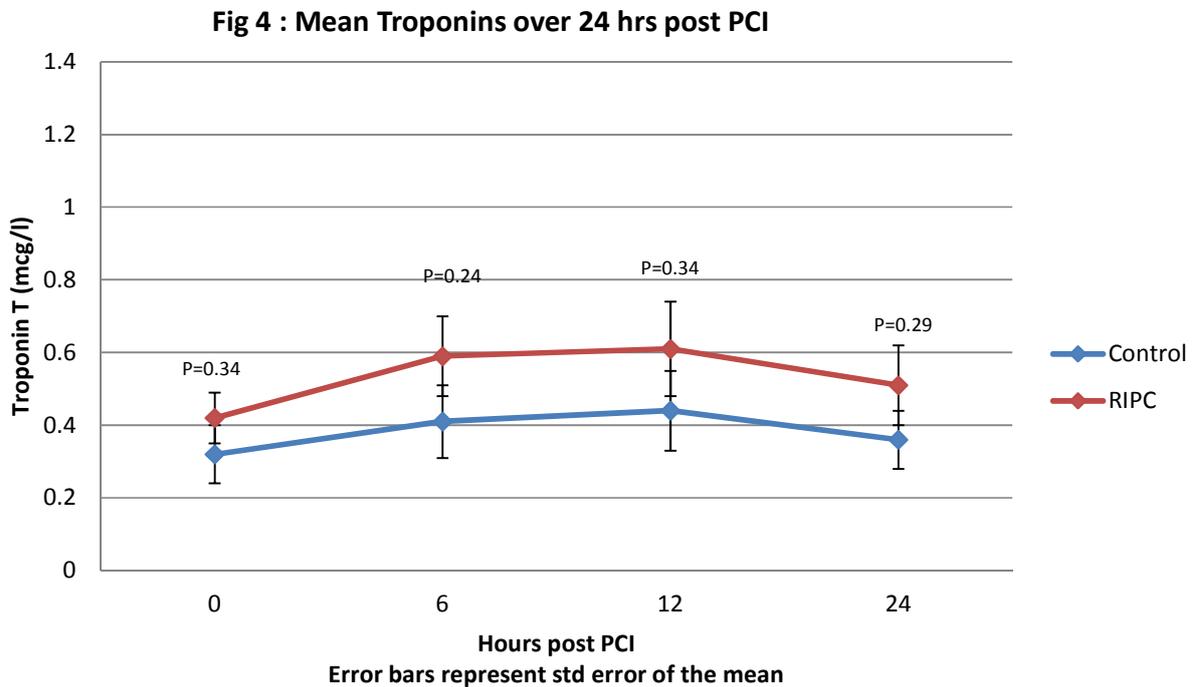
There was no statistical difference in the cTnT levels, at any time intervals post procedure between the two groups. The mean baseline troponin levels (0 hour troponins) of RIPC group was higher than that of the control group (0.42(±0.42) in RIPC group vs. 0.32(±0.46) in control group). This baseline inequality leads to the appearance, in the above graph, as higher Troponin release at individual time points in the RIPC group. However, the Troponin area under the curve (AUC) over 24 hours adjusted to the baseline Troponin levels, showed no statistical difference between the two groups (Mean ±SD of Troponin AUC in RIPC group 13.5±15.2 vs. 11.96±13.05 in control group, p =0.64).

3.3.5 Periprocedural Myocardial Infarction Incidence (PMI)

The incidence of PMI was calculated and compared between the two groups, and the results are summarised in the table 3.7. The new Universal definition of myocardial infarction, defines peri-procedural myocardial infarction during PCI as an increase in cardiac enzymes more than 3 times ULN. In patients with already increased baseline troponins, further raise

in Troponins should be more than 20% of the baseline troponin levels. Therefore, in this NSTEMI cohort an increase in baseline Troponins more than 3 x ULN (> 0.03µg/L) and greater than 20% of the baseline (0 hour Troponins) were taken as PMI.

Table 3.7

PMI definition	Control (n=31)	RIPC (n=35)	P value
>0.03µg/L increase and >20% increase in cTnT level from baseline (0 hour) level	16 (51.6%)	20 (57.1%)	0.65
CKMB mass increase >12µg/L (>3x ULN) from baseline level and >20% increase from the baseline level	2 (6.5%)	8 (22.9%)	0.09

There was no statistically significant difference in PMI incidence between the two groupswhen the increase in Troponin levels was compared according to the new universal definition of MI (51.6% in control group vs. 57.1% in RIPC group, p = 0.65). However, when the same criteria of PMI definition was applied to CKMB marker (> in 3x ULN plus 20% increase in the enzyme levels from baseline levels), a higher number of patients in the RIPC group had PMI, which was nearly statistically significant (6.5% in control group vs. 22.9% in the RIPC group).

3.4. DISCUSSION

In this prospective, two-Centre open label randomized controlled trial, RIPC did not demonstrate a statistically significant benefit in the reduction of peri-procedural myocardial injury, determined by serial myocardial enzymes post percutaneous coronary interventions, in high risk patients. RIPC did not demonstrate reductions in the incidence of peri-procedural myocardial infarction either. In elective PCI settings, Hoole *et al.*(268) have demonstrated a significant peri-procedural myocardial enzyme reduction in their randomised controlled trial with similar RIPC protocol. A previous smaller study by Ilidromitis *et al.* (269), had failed to demonstrate any improvement in cardiac enzymes in single vessel disease patients undergoing elective PCI. There are several reasons, why our study with RIPC intervention fails to demonstrate significant benefit in myocardial enzyme release, compared to placebo. These issues will be further elaborated in this section.

Firstly, our study is underpowered for the primary end point of 20% absolute reduction in the incidence of PMI, with 80% power and significance of 0.05. While calculating power, it was estimated that 93 patients in each group would be needed to reach the 80% power for the study. We estimated 1/3 of the patients undergoing urgent in-patient coronary angiography would not proceed for PCI, and therefore, estimated that a total patient cohort of 290 would be sufficient to reach the necessary power. However, in our study, 2/3 of the enrolled patients (n=153) did not proceed to PCI, with only 101 patients completing the study. Therefore, although pre-specified total number of patients based on intention to treat analysis (n=293) was achieved, the number of patients completing the study were insufficient to reach a negative conclusion from this intervention. In a positive clinical study, even if the study is underpowered, it is generally accepted to conclude in favor or against the hypothesis. However, in a negative study, reaching the pre-specified power is very important to conclude that the intervention is not different from the control group, with respect to a primary end point. Consequently, to reach the pre-specified power in our study, we have calculated that a further 250 patients would need to be recruited, to achieve a total of 186 patients, who will have completed the study after exclusions. To this end, we have obtained

further amendments from the joint UCLH/UCL ethics committee, and are continuing to recruit patients in order to reach the pre-specified power.

Secondly, the patient groups we studied are the patients presenting acutely with NSTEACS. A very valid possibility in these patients is that, the episode of NSTEMI/UA itself can provide a strong preconditioning stimulus, directly on the heart. There are however, no studies demonstrating this effect, as predicting an ACS event is any population is impossible.   The unstable thrombus in the denuded plaque is a source of recurrent ischaemia in these patients, which theoretically should precondition the heart directly. Consequently, the RIPC stimulus prior to the PCI procedure, may not have contributed further to the already preconditioned hearts in this group of patients.

The mean duration from the last chest pain to PCI was around 3 days in both groups. Even if the direct myocardial preconditioning effect from the initial ischaemic event has disappeared, the myocardium should be ideally in the phase of second window of preconditioning during this period (3 days)(19). Therefore, RIPC stimulus is unlikely to have had a further protective effect in our cohort of patients. In elective PCI settings, the myocardium would not have had any recent ischaemic episode contributing for inherent preconditioning, and consequently neither any effect of second window of protection. Therefore, RIPC in this group of patients could be cardioprotective, and consequently decrease the peri-procedural myocardial injury. This probably explains why, in our study, we failed to demonstrate even a trend towards the benefit of enzyme reduction in the intervention group, whereas other investigators who studied RIPC in elective angioplasty settings, could show significant protection(163).

Thirdly, although most of the pre-procedure variables were comparable between the two groups, a significantly higher proportion of patients in the control group had pre-existing diagnosis of angina prior to enrolment (45% in control gp vs. 25% in RIPC gp, p= 0.045). As angina is a known clinical ischaemic preconditioning stimulus by itself (331, 332), it is possible that these patients with angina in the control group are already in a cardioprotected state. This certainly could have contributed to the diminished protective effect in the intervention group, by decreasing the myocardial injury in the control group. A one way

between-subjects ANOVA test to determine the relationship between Troponin release (AUC) and angina as the independent factor, showed near significant results (mean Tr-AUC 6.57 in patients with angina vs. 10.64 in patients without angina, $p= 0.09$), prompting the theory that previous angina could be a strong cardioprotective confounder. However, even after adjusting this confounding effect in general linear univariate model (GLM) to our study, RIPC still did not have any significant reducing effect on Tr-AUC. (Mean Tr-AUC control = 8.84 vs. 7.4 in RIPC, $p= 0.68$).

Fourth, in our study, the average number of balloon inflations during PCI in both groups is around 7 inflations. Balloon inflations contribute to brief ischaemia until the balloon is deflated. 7 balloon inflations appear to be much higher than the average number of inflations found in typical PCI. This, high number of balloon inflations in our study groups may have contributed to some preconditioning effect in the control group too, which could have, therefore, lowered enzyme release in the control group, masking the true beneficial effect of remote preconditioning. The balloon inflations were, however, typically less than 30 seconds, and the preconditioning effect of these inflations might not be significant.

Finally, in our study we did not assess the myocardium at risk though any angiographic methods or scores. There are various scoring systems (e.g. jeopardy score) based on vessel to be intervened and presence of collaterals through which relative myocardium risk could be estimated. Because, in our study we have not estimated this, any difference in myocardium risk between control and RIPC groups remains unknown, and potentially there could be an undetermined confounder. However, there were no differences in other angiographic variables between the two groups, including type of vessel intervention, stent length, stent diameter etc.

Therefore, our study has several limitations, which could explain lack of benefit from RIPC in the PCI settings. These limitations are likely to prevail in most of the proof of concept studies with limited number of patients. A large multicentre randomised controlled trial, with hard clinical end points is the best way to determine if RIPC is beneficial in urgent PCI settings. A large RCT is needed, incorporating both elective and urgent PCIs, especially due to clear benefits seen in the CRISP study (268) in elective PCI settings, and absence of any trend towards benefit in our study. The similar findings in a large multicentre study could validate

some of the important reasons for lack of benefit in urgent PCI settings, that we discussed above.

One very interesting observation from our study is that, although the cardiac biomarkers cTnT and CK-MB release post-PCI do show a strong correlation, as one would expect, the relative proportional increases are not similar. Therefore, the incidence of PMI when one biomarker is used appears significantly different in the same group when the other biomarker is used to define PMI. In our cohort, cTnT increase >1 ULN occurred in 61% of the patients, >3 ULN occurred in 55% of the patients. However, CKMB increase >1 ULN occurred in only 33% of the patients, and >3 ULN occurred in only 11 % of the total cohort. Although the Joint ESC/AHA/ACC universal definition of MI advocates the use of either cardiac enzyme (Troponins or CKMB) in determining the occurrence of PMI, certainly it appears that the incidence in PMI would differ significantly in different studies, based on which cardiac biomarker is used to diagnose the PMI.

4. REMOTE ISCHAEMIC PRECONDITIONING IN TYPE 2 DIABETIC PATIENTS UNDERGOING CORONARY ARTERY BYPASS SURGERY

Remote Ischaemic Preconditioning has an important application in the field of cardiovascular surgical settings. Preconditioning in myocardial ischaemia reperfusion injury is a well established cardioprotective intervention, as discussed in chapter 1. During cardiac surgeries, cross clamping the aorta for the blood-less field, and releasing the cross clamp after surgery mimics a typical myocardial ischaemia reperfusion setting, albeit, the myocardial oxygen demand during these procedures are reduced by administration of cardioplegic solution and other techniques, which have been elaborately discussed in chapter 1. In fact, the first ever human study looking at the benefit from remote preconditioning was conducted by Cheung *et al.* (333), in the context of cardiac surgery among a paediatric group of patients undergoing corrective surgery for congenital heart defects. Yellon's group from the Hatter Cardiovascular Institute, was the first to show the peri-operative myocardial enzyme reduction in adult patients undergoing elective CABG in a group of 56 patients (334). This study included both diabetic and non-diabetic patients. They also showed, in a separate study of non- diabetic patients undergoing elective CABG and receiving cardioplegic method of surgical myocardial protection, that RIPC reduces perioperative myocardial enzyme release by 40% (270). In the animal studies, the diabetic hearts have been shown to have varied susceptibility to ischaemic injury and in their preconditioning ability. In summary, it appears that the diabetic hearts from animal models in particular circumstances, are more resistant to ischaemic injury, and although further reduction of this IR injury is possible through preconditioning, it requires an increased preconditioning stimulus. This topic has been discussed elaborately in chapter 1 (see sections 1.13.1 and 1.13.2). The aim of this study was to specifically study whether RIPC reduces peri-operative myocardial injury in type-2 diabetic patients undergoing CABG.

METHODS

4.1.1 Overview

We conducted a two Centre open label randomized controlled study, where we studied the effect of remote ischaemic preconditioning induced by transient limb ischaemia on peri-operative myocardial injury in type 2 diabetic patients undergoing elective CABG with or without concomitant aortic valve surgery.

4.1.2 Ethical Approval and Informed Consent

The protocol for this study was written in accordance with the international Conference on Harmonisation- Good Clinical Practice (ICH-GCP) guidance, and the study was subject to approval by the joint University College London (UCL)/University College London Hospitals (UCLH) Committees for the ethics of human research. Approval for the study was sought on the standard application form, initially provided by NHS COREC (Central office for the Research Ethics Committees), and subsequently integrated with the National Research Ethics Service (NRES), affiliated to the National Patient Safety Agency. In addition to the study protocol, the patient information sheet, consent form, and letter to the patient's general practitioner were also approved by the ethical committee.

Once ethical approval was obtained, a separate application was made to the research and development department UCLH, who also acted as sponsors of the study. Any amendments to the protocol were classed as major or minor, as per advice from the ethical committee. I made the amendments and submitted the documents, annotated with appropriate dates and version numbers, for approval of each amendment. An Integrated Research Application System (IRAS) form, and a non-NHS SSI application (no: 26244) were filled and transferred out to seek approval from HCA ethics committee to include second recruitment centre -The Wellington Hospital.

Any serious adverse event or reaction during the course of the study had to be reported to the R&D department as per guidelines. No serious adverse events or reactions occurred during the course of this study.

Type 2 diabetic patients admitted for CABG with or without concomitant aortic valve surgery were approached on the day prior to their scheduled surgery date, and were explained about the study in detail, and their willingness to participate in the trial was established. Informed consent was obtained using the current version of the patient information sheets and consent forms, and patients' signatures obtained. A copy of the signed consent form was given to the patient with the information sheet and the originals filed in the hospital notes. An additional copy of the signed consent form was filed at the Hatter Cardiovascular Institute for patients recruited at the Heart Hospital, and at the Wellington Hospital for patients recruited at the Wellington Hospital site.

4.1.3 Patient Recruitment

Consecutive Type 2 diabetic patients admitted for CABG between April 2008 and September 2010, were screened for recruitment into the study at the Heart Hospital site. At the Wellington Hospital site, the patient recruitment was started from March 2010. The first 8 patients in this study at the Heart Hospital site, were recruited by Dr Vinod Venugopal, and I continued patient recruitment from October 2008.

4.1.4 Inclusion Criteria

Age > 18 years.

Consecutive type-2 diabetic patients undergoing elective coronary artery bypass surgery with or without concomitant aortic valve repair/replacement. Diabetes was confirmed to be diagnosed by GP or a hospital specialist and complied with 2006 WHO criteria of diagnosing diabetes.

WHO criteria for diagnosing diabetes mellitus (2006)

1. Diabetes symptoms (ie polyuria, polydipsia and unexplained weight loss) plus

- a random venous plasma glucose concentration $\geq$ 11.1 mmol/l
  or

- a fasting plasma glucose concentration $\geq$ 7.0 mmol/l (whole blood $\geq$ 6.1mmol/l) or
- two hour plasma glucose concentration $\geq$ 11.1 mmol/l two hours after 75g anhydrous glucose in an oral glucose tolerance test (OGTT).

2. With no symptoms diagnosis should not be based on a single glucose determination but requires confirmatory plasma venous determination. At least one additional glucose test result on another day with a value in the diabetic range is essential, either fasting, from a random sample or from the two hour post glucose load. If the fasting or random values are not diagnostic the two hour value should be used

4.1.5 Exclusion Criteria

Renal failure. (EGFR <35 ml/min/1.73m^2)

Peripheral vascular disease, affecting the upper limbs.

Recent myocardial infarction (STEMI or NSTEMI in the immediate four weeks before CABG).

Angina within three days before the surgery.

Patients on Nicorandil or Glibenclamide.

Serum Troponins (cTnT) remain elevated for up to two weeks after a myocardial infarction. Moreover, some patients undergo CABG surgery as an urgent procedure following an acute myocardial infarction (AMI). We, therefore, excluded patients who had an AMI within four weeks immediately prior to the cardiac surgery. Likewise, since the kidneys excrete cTnT, the excretion of this marker would be unreliable in renal failure patients, and in these group of patients, the baseline levels of cTnT can be elevated. Therefore, these patients were excluded from the study.

4.2.1 Anaesthetic Procedure

Pre-medication comprising Temazepam 10-20mg orally was administered to each patient one hour before surgery. On arrival to the anaesthetic room, a peripheral venous cannula was inserted and the patients were sedated with Midazolam given intravenously. An arterial cannula was inserted prior to the induction of anaesthesia for invasive monitoring of the blood pressure. Continuous arterial pressure monitoring was commenced and an infusion of normal saline started. Anaesthesia was induced with Midazolam $\pm$ Etomidate or Propofol, Fentanyl (5-15μg/kg) and either Pancuronium (0.1mg/kg). The trachea was intubated, mechanical ventilation commenced with $O_2\pm$ air, and anaesthesia was maintained by either halogenated anaesthetics (Isoflurane or Sevoflurane) or with an infusion of Propofol, administered by target controlled infusion to achieve a target plasma concentration of 3-8μg/ml. Midazolam, Fentanyl, and Pancuronium were given as required. Arterial blood pressure, central venous pressure, electrocardiogram and nasopharyngeal temperature were recorded continuously.

4.2.2 Randomisation and Transient Limb Ischaemia Protocol

Patients were randomised to either control group or RIPC group, using an electronic randomisation method of random sequence generator for two treatments. A simple randomisation method was used to allocate patients to receive either RIPC or the placebo. http://www.random.org/sequences/

RIPC was induced by transient limb ischaemia in patients randomised to receive the intervention. This was achieved by inflating a standard 9 inch blood pressure cuff to 200 mmHg on the upper arm for five minutes for three cycles, each separated by a reperfusion period of five minutes during which time the cuff was kept deflated.

The protocol was applied to the right arm whenever possible, but if the arterial cannula for invasive blood pressure monitoring was inserted in the right radial artery, the RIPC protocol was applied on the left arm. The protocol was commenced after the patient was anaesthetised to avoid any discomfort.

4.2.3 Surgical Procedure

After sternotomy, the left internal mammary artery (LIMA) was dissected down from the anterior chest wall, while the great saphenous vein was harvested from the leg. Aortic root, and the right atrial appendage were inserted with bypass cannulae and a standard non-pulsatile cardiopulmonary bypass (CPB) was established using the membrane oxygenator.

The coronary artery bypass grafts (LIMA or saphenous venous grafts) were constructed on cardiopulmonary bypass, with each distal anastomosis to the coronary arteries being performed during cardiac standstill. This was achieved by cross clamping the aortic root, accompanied by either ventricular fibrillation, or by the injection of cardioplegic solution. Moderate hypothermia, between 28 and 32℃, was induced by using the cardiopulmonary bypass circuit and, if required, an additional ice pack around the heart in the pericardial sac.

In the technique of cross clamp fibrillation, the aorta was cross clamped and an alternating current was applied to the epicardium to induce ventricular fibrillation, afterwhich the distal anastomosis was performed. After each distal anastomosis, the aortic clamp was released and a direct current shock used to defibrillate the heart. The proximal anastomoses were performed after all the distal anastomoses, with the heart still beating.

In the second technique, a cardioplegic solution comprising of 1 part of St Thomas Hospital Cardioplegia solution 1 mixed with 4 parts of cold blood, was injected antegradely into the aortic root, and the distal anastomoses were performed during the duration of a single cross-clamp. An additional bolus of cardioplegia solution was given into the vein graft following each distal anastomosis. In cases of completely occluded coronary arteries, the cardioplegic solution was injected retrogradely into the coronary sinus. Thus, depending upon the choice of the operating surgeon, either the technique of cross-clamp fibrillation or cardioplegia was used during the surgery.

Re-warming was commenced at the time of the last proximal anastomosis, and the cardiopulmonary bypass was discontinued. Protamine was used to reverse the effect of heparin.

4.3.1 SerumTtroponin-T Measurement

Blood samples for the measurement of serum cTnT were taken pre-operatively, and at 6, 12, 24, 48, and 72 hours following surgery. cTnT was measured quantitatively by one-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010; Roche). The lower detection limit of this assay (99th percentile URL) is 0.01 μg/L with a co-efficient of variation of 10%, and recommended diagnostic range of 0.03-0.09 μg/L, indicating possible myocardial injury, and a threshold of ≥ 0.1μg/L, indicating myocardial injury suggestive of myocardial infarction.

The area under the curve indicative of absolute Troponin release over 72 hours was calculated using the trapezoid rule, with the formula below;

$$AUC_{t1-t2} = [(cTnT \text{ at } t1 \text{ hours} + cTnT \text{ at } t2 \text{ hours})/2] \times (t2-t1)$$

$$AUC \ 72 \text{ hours} = AUC_{0-6} + AUC_{6-12} + AUC_{12-24} + AUC_{24-48} + AUC_{48-72}$$

4.3.2 Assessment of Immediate Clinical Outcomes

During the post-operative period, data were recorded including the details of postoperative intensive care unit parameters, such as duration of ventilation, inotrope use, renal function and urine output, duration of hospital stay and post-operative arrhythmias.

4.3.3 Statistical Analysis

Standard statistical methods were used for analysis. Histograms with normal plots were performed to check that the data in the two groups was normally distributed. Differences between the groups for continuous variables were tested using t-test and for categorical variables using the Chi-square test. The general linear multivariate model was used to test multivariate analysis. Significance was interpreted at the 95% confidence interval having corrected for any inequality of variance between groups. Data was analysed using SPSS statistical software version 16.

4.3.4 Sample Size Calculation

Our Institute's previous clinical study involving RIPC in CABG settings, which included both non-diabetic and diabetic patient cohorts, and which was the first ever proof of concept study of RIPC in the clinical settings formed the basis of the sample size in this study (79). Based on this study, we determined that we would require a sample size of 23 patients in each group (total 46 patients) to detect a reduction in absolute Troponin release (AUC 72 hours) of 15μg/L, with a standard deviation of 18μg/L, and a power of 80% at the two sided 5% significance level.

4.4 RESULTS

A total of 72 consecutive Type 2 diabetic patients were approached over a two year period, who fulfilled the inclusion criteria for this study. Six patients refused to take part in the study, 4 patients had their baseline pre-operative eGFR < 35mls/min/1.73m^2, and had to be excluded from further research protocol. One patient underwent off-pump bypass surgery and had to be excluded from further analysis. A total of 61 patients, therefore, completed the research study over a two year period. Thirty patients were in the RIPC group, and remaining 31 patients were in the control group. The flow chart below shows the schematics of patient screening and recruitment.

Fig 5
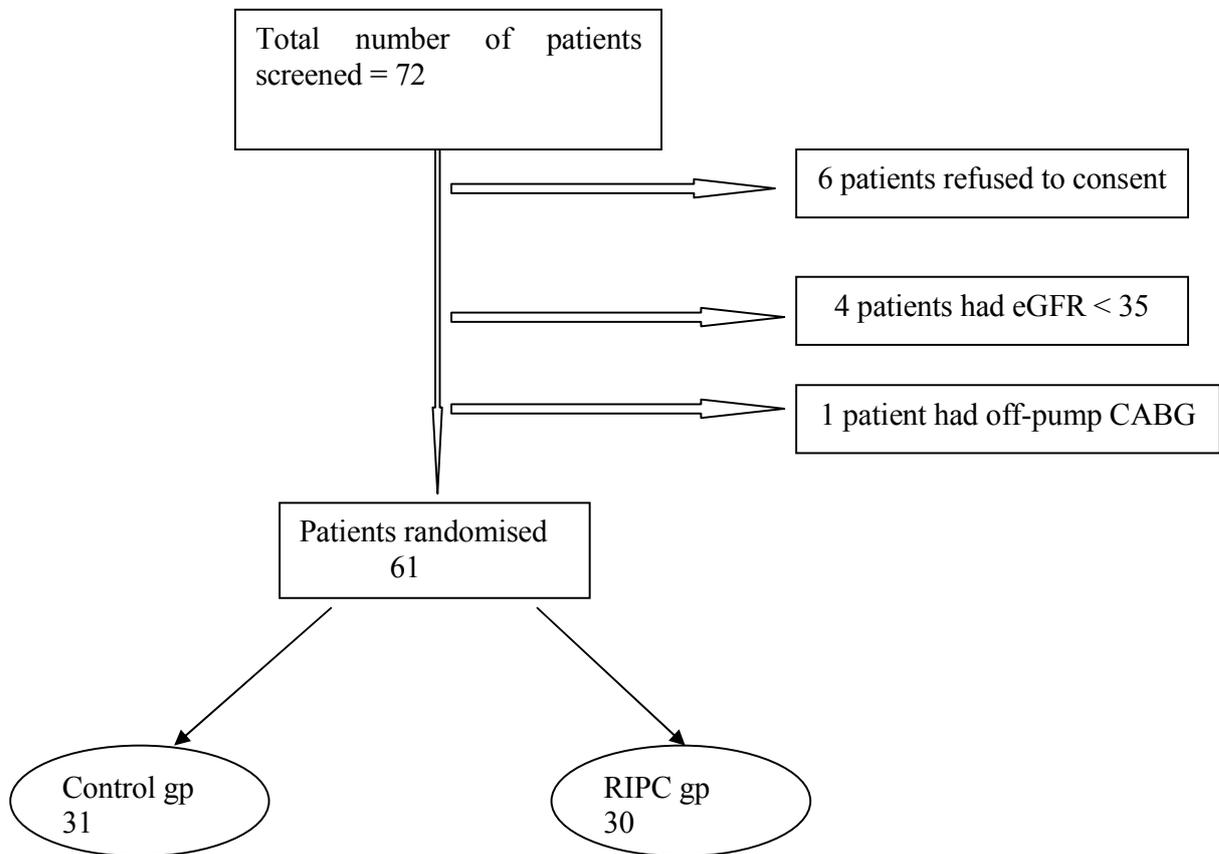
4.4.1 Baseline Characteristics- Patient Profile

The baseline characteristics of the patients are summarised in table 4.2. Both groups matched well, and had no significant differences with respect to most of the baseline characteristics. The control group had higher mean HbA1C level compared to the RIPC group (8.52 vs. 6.75, p = 0.003).

4.4.2 Pre-Procedure Drug Profile

The control group had higher proportion of patients on insulin compared to RIPC group (38.7% vs. 16.7%, p = 0.05). There were, however no significant differences in other drug profile, prior to the procedure between the two groups. The drug profile of both groups has been summarised in table 4.3.

Table 4.2

Demographics	Control (n=31)	RIPC (n=30)
Age (years)	67.0 ($\pm$7.5)	69.3 ($\pm$10.3)
Male	22 (71%)	21 (70%)
Female	9 (29%)	9 (30%)
Hypertension	28 (90.3%)	25 (83.3%)
Dyslipidaemia	24 (77.4%)	27 (90%)
Previous MI	12 (38.7%)	12 (40%)
Previous CVA	2 (6.5%)	3 (10%)
Current smoker	4 (12.9%)	1 (3.8%)
Ex Smoker	15 (48.4%)	10 (38.5%)
Never smoked	12 (38.7%)	15 (58.7%)
Family History	10 (33.3%)	5 (16.7%)
Body surface Area (m^2)	1.91 ($\pm$0.18)	1.86 ($\pm$0.17)
Body Mass Index (BMI)	29.9 ($\pm$6.0)	29.3 ($\pm$4.1)
Hb A1c level	8.52 ($\pm$1.8)	6.75 ($\pm$0.95)

Table 4.3

Drugs	Control	RIPC
Aspirin	20 (64.5%)	13 (43.3%)
Beta Blocker	21 (67.7%)	18 (60%)
Statin	27 (87.1%)	27 (90%)
ACE inhibitor	23 (74.2%)	23 (76.2%)
Ca^{2+} channel blocker	10 (47.6%)	10 (38.5%)
Nitrate	9 (29%)	10 (33.3%)
Insulin	12 (38.7%)	5 (16.7%)
Suphonylurea	11 (35.5%)	7 (23.3%)
Metformin	20 (64.5%)	19 (63.3%)

4.4.3 Pre-operative Clinical Parameters.

Both groups matched well with respect to pre-operative LV function, Left main stem involvement, EuroSCORE, and Parsonnet scores. EuroSCORE (**Euro**pean **S**ystem for **C**ardiac **O**perative **R**isk **E**valuation) and Parsonnet scoring systems are logistic risk assessment models, used to predict the early operative mortality among patients undergoing CABG. The EuroSCORE in both groups in our cohort was more than 3 and less than 6, which translates into a mortality risk of 3% and therefore confirms that our patient cohort comprised of moderate-risk patients.

Table 4.4

Pre-operative clinical parameter	Control	RIPC
LV ejection Fraction Good (>55%) Fair (35-55%) Poor (<35%)	 20 (71.4%) 8 (28.6%) 0	 19 (73.1%) 7 (26.9%) 0
Left Main Stem (LMS) > 50% disease	3 (10.7%)	3 (11.5%)
EuroSCORE	4.16 (±2.7)	3.73 (±2.3)
Parsonnet Score	13.38 (±7.5)	14.23 (±8.5)

4.4.4 Intra-operative Parameters

The intra-operative variables in the two patient groups are summarised in table 4.5. Antegrade intermittent cold blood cardioplegia was the predominant method of myocardial preservation strategy used during cardiac surgery in this study cohort (81%). Intermittent cross clamp fibrillation was used in 19% of the patients as myocardial preservation strategy. All intra-operative variables were evenly matched between the two groups. Both groups had equal numbers of patients undergoing concomitant aortic valve replacement (4 in each group) with CABG.

Table 4.5

Operative parameters	Control	RIPC
Bypass temperature	32.3^0 ($\pm$1.2)	32.5^0 ($\pm$1.3)
Type of cardioprotection		
Cardioplegia	26 (83.9%)	24 (80%)
Cross clamp fibrillation	5 (16.1%)	6 (20%)
Concomitant aortic valve surgery	4 (12.9%)	4 (13.3%)
No of vessels bypassed (mean)	2.74 ($\pm$0.89)	2.80 ($\pm$0.80)
Cross clamp time(min)	56.68 ($\pm$23.0)	51.67 ($\pm$23.3)
Cardiopulmonary bypass time (CPB) (min)	86.77 ($\pm$32.1)	82.47 ($\pm$24.1)
Maintenance anaesthesia Isoflurane	23 (74.2%)	25 (83.3%)
Sevoflurane	8 (25.8%)	5 (16.7%)
No of Grafts		
One	3 (9.7%)	3(10%)
Two	7 (22.6%)	4 (13.3%)
Three	17 (54.8%)	19(63.3%)
More than three	4 (12.9%)	4 (13.3%)

4.5.1 Serum Troponin Release

Cardiac TnT levels were measured pre-operatively at the time of anaesthetic induction, and at 6, 12, 24, 48, and 72 hours post cardiac surgery. The mean ($\pm$SD) cTnT levels at these time points are shown in table 4.6.

Table 4.6

Time (Hours)	Control	RIPC	Mean difference	Confidence interval	P Value
6	0.59±0.37	0.54±0.41	0.05 ±0.10	-0.15 - 0.25	0.62
12	0.51±0.23	0.46±0.31	0.042±0.07	-0.10 - 0.18	0.55
24	0.38±0.21	0.35±0.20	0.029±0.05	-0.07 - 0.13	0.58
48	0.27±0.16	0.24±0.14	0.035±0.03	-0.04 - 0.11	0.38
72	0.23±0.17	0.22±0.15	0.014±0.04	-0.07 - 0.09	0.73

Values present mean ± SD (in μg/L)

There were no statistical differences in the mean cTnT levels between the RIPC group and control groups at 6, 12, 24, 48 or 72 hours. There was no significant difference in the cTnT AUC over 72 hours between the two groups. The mean cTnT AUC in the control group was 24.55 (±13.13) μg/L.72 hrs vs. 22.38(±13.39) μg/L.72 hrs in RIPC group, p=0.52. There was no statistical difference in the maximum Troponin level over 72 hours between the two groups, maximum cTnT control group 0.64 (±0.34) μg/L vs. 0.57(±0.40) μg/L in the RIPC group, p = 0.51.

Fig 6: Troponin release post CABG 1
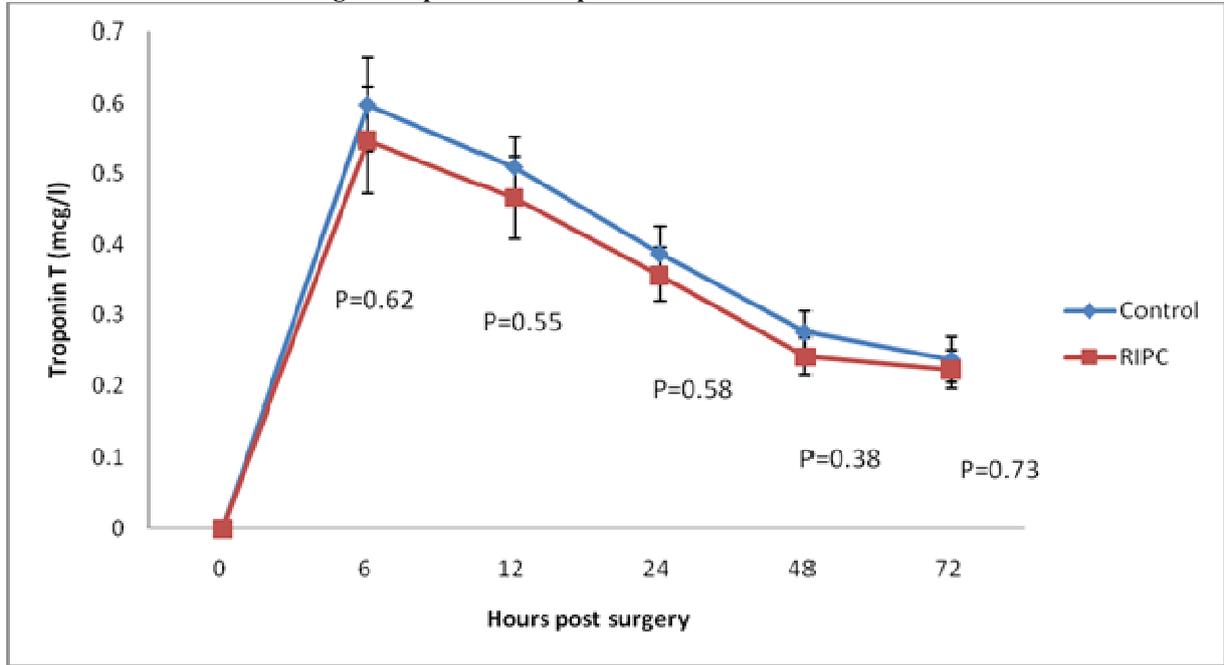
Error bars in the figure represents Std Error of the mean

4.5.2 Serum CK-MB Release

Serum CK-MB mass were measured pre-operatively at the time of anaesthetic induction and at 6, 12, 24, 48, and 72 hours post cardiac surgery. The mean ($\pm$SD) CK-MB levels at these time points are shown in table 4.7.

Table 4.7

Time (Hours)	Control	RIPC	Mean difference	Confidence interval	P Value
0	3.40$\pm$0.74	3.72$\pm$2.07	0.27$\pm$0.47	-0.12 - 0.68	0.56
6	23.95$\pm$11.7	23.32$\pm$9.5	0.62 $\pm$3.1	-5.78 - 7.04	0.84
12	20.07$\pm$9.4	17.71$\pm$6.8	2.3$\pm$2.46	-2.61- 7.33	0.34
24	16.63$\pm$9.4	15.46$\pm$6.6	1.17$\pm$2.37	-3.61 - 5.96	0.62
48	7.32$\pm$3.12	7.34$\pm$3.85	0.025$\pm$1.07	-2.19 - 2.14	0.98
72	5.07$\pm$3.4	4.49$\pm$1.5	0.57$\pm$0.81	-1.06 – 2.22	0.48

Values present mean $\pm$ SD (in µg/L)

There were no significant differences in the CK-MB mass between the two groups at any time points postoperatively, i.e. at 6, 12, 24, 48 or 72 hours. In addition, there was no statistical difference in the peak CK-MB mass levels over 72 hours between the two groups, peak CK-MB mass levels in the control group was 24.83 ($\pm$12.3) μg/L vs. 23.66 ($\pm$9.4) μg/L in the RIPC group, p = 0.71.

4. 5.3 Post-operative Ventilation Times

Post-operative ventilation times did not differ significantly between the two groups. Mean ventilation time for the control group was 7.74 ($\pm$5.47) hours vs. 7.93 ($\pm$3.49) hours in the RIPC group, p = 0.87.

4.5.4 Post-operative ITU Stay and High-care Stay

There were no significant differences in the duration of ITU stay between the two groups post-operatively. In addition, there was no statistical difference in the duration of high-care stay between the two groups. Mean ITU stay (number of nights) in the control group was 2.77 ($\pm$4.6) vs. 2.8($\pm$2.4) in the RIPC group, p= 0.98. Mean number of days of high care stay in the control group was 3.68 ($\pm$4.5) vs. 4.69 ($\pm$6.1) days in the RIPC group, p = 0.47.

4.5.5 Postoperative Inotrope Requirement and Inotrope Score

Data were obtained regarding the inotrope requirement post-operatively in all patients during their ITU stay. Based on the maximum inotrope concentration required, inotrope-score was calculated using the formula: Inotrope score = Dosages (in μg/kg/min) of Dopamine + Dobutamine + [(Adrenaline + Noradrenaline + Isoproterenol) × 100] + [Enoximone × 15], adapted from Ko *et al.*(335).

Inotrope support was required in 5 (16.1%) of patients in the control group compared to 11 (36.6%) patients in the RIPC group. This difference, however, was not statistically significant,

p= 0.12. The inotrope scores among the patients requiring inotrope support did not differ significantly between the two groups. Inotrope score in the control group was 7.40 ($\pm$5.2) µg/kg/min vs. 9.98 ($\pm$8.67) µg/kg/min in the RIPC group, p = 0.55.

4.5.6 Post-operative Atrial Fibrillation (AF)

New AF occurred in 6 (19.4%) patients in the control group vs. 4 (13.3%) patients in the RIPC group. This was not statistically significant, p= 0.73.

4. 5.7 Post-operative Acute Kidney Injury (AKI)

AKI was defined as an abrupt (within 48 hours) reduction in kidney function either with an absolute increase in serum creatinine of more than or equal to 0.3 mg/dl ($\geq$ 26.4 µmol/l), a percentage increase in serum creatinine of more than or equal to 50% (1.5-fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5 ml/kg per hour for more than six hours) (336).

AKI was observed in 4 (12.9%) patients in the control group vs. 2 (6.7%) patients in the RIPC group. This difference was not statistically significant, p = 0.67.

4.6 Sub-group Analysis of Patients Undergoing Cardiac Surgery with Cardioplegia as Myocardial Preservation

As described in previous chapters, 85% of the cardiac surgeons in the UK use cardioplegia as myocardial preservation strategy. Cross clamp fibrillation is an alternative myocardial preservation strategy, invariably involving less aortic cross clamp time, however, devoid of additional protection from cardioplegic solution. Extensive literature reviews have concluded that both these myocardial preservation strategies provide equivalent cardioprotection during cardiac bypass surgeries (192). Previous studies evaluating the invasive ischaemic preconditioning (IPC), have consistently shown benefit in cardiac surgery employing cross clamp fibrillation as myocardial preservation strategy (262, 263). However,

in studies involving cardioplegia as myocardial preservation strategy, the beneficial effect of IPC has been controversial (265-267). It is, therefore, important in evaluating RIPC, that we also analyse a cohort of our patients employing cardioplegic myocardial preservation.

50 patients (82%) of our cohort had cardioplegia as myocardial preservation strategy. These patients were further analysed grouping them into controls and the RIPC group, and the results are detailed below. 26 patients were in the control group and 24 patients were in the RIPC group.

4.6.1 Baseline Characteristics- Patient Profile

The baseline characteristics of the patients are summarised in table 4.8. Both groups matched well and had no significant differences with respect to most of the baseline characteristics. The control group had higher mean HbA1C level compared to the RIPC group (8.67 vs. 6.62, p = 0.002).

4.6.2 Pre-Procedure Drug profile

The control group had higher proportion of patients on insulin compared to RIPC group (38.5% vs. 12.5%, p = 0.05). The control group also had a higher proportion of patients on Aspirin pre-operatively compared to the RIPC group (65.4% vs. 37.5% p = 0.05). There were, however, no significant differences in other drug profiles prior to the procedure between the two groups. The drug profile of both groups has been summarised in table 4.9.

Table 4.8

Demographics	Control (n=26)	RIPC (n=24)
Age (years)	67.4 (+6.9)	68.8 (+10.5)
Male	20 (76.9%)	16 (66.7%)
Female	6 (23.1%)	8 (33.3%)
Hypertension	23 (88.5%)	20 (83.3%)
Dyslipidaemia	19 (73.1%)	21 (87.5%)
Previous MI	10 (38.5%)	7 (29.2%)
Previous CVA	2 (7.7%)	2 (8.3%)
Current smoker	3 (11.5%)	1 (4.5%)
Ex Smoker	13 (50%)	8 (36.4%)
Never smoked	10 (38.5%)	13 (59.1%)

Family History	8 (32%)	4 (16.7%)
Body surface Area (m^2)	1.93 (±0.18)	1.86 (±0.17)
Body Mass Index (BMI)	30.6 (±6.0)	29.6 (±4.1)
Hb A1c level	8.67 (±1.8)	6.62 (±0.96)

Table 4.9

Drugs	Control	RIPC
Aspirin	17 (65.4%)	9 (37.5%)
Beta Blocker	16 (61.5%)	16 (66.7%)
Statin	273(88.5%)	21 (87.5%)
ACE inhibitor	19 (73.1%)	18 (75%)
Ca^{2+} channel blocker	8 (47.1%)	8 (40%)
Nitrate	7 (26.9%)	7 (29.2%)
Insulin	10 (38.5%)	3 (12.5%)
Suphlonylurea	9 (34.6%)	5 (20.8%)
Metformin	17 (65.4%)	13 (54.2%)

4.6.3 Pre-operative Clinical Parameters.

Both the groups matched well with respect to pre-operative LV function, Left Main Stem involvement, EuroSCORE, and Parsonnet scores. The EuroSCORE in both the groups in our cohort was more than 3 and less than 6, which translates into mortality risk of 3% and, therefore, confirms that our patient cohort comprised of moderate-risk patients.

Table 4.9.1

Preoperative Clinical parameters	Control	RIPC
LV ejection Fraction Good (>55%) Fair (35-55%) Poor (<35%)	16 (30.4%) 7 (69.6%) 0	15 (28.6 %) 6 (71.4 %) 0
Left Main Stem (LMS) > 50% disease	3 (12.5%)	2 (9.1%)
EuroSCORE	4.27 (±2.8)	3.79 (±2.5)
Parsonnet Score	14.08 (±7.7)	14.76 (±8.9)

4.6.4 Intra-operative Parameters

The intra-operative variables in the two patient groups are summarised in table 4.9.2. All intra-operative variables were evenly matched between the two groups. Both groups had equal number of patients undergoing concomitant aortic valve replacement (4 in each group) with CABG.

Table 4.9.2

Operative parameters	Control	RIPC
Bypass temperature	32.1^0 (±1.1)	32.2^0 (±1.2)
Concomitant Valve Surgery	4 (15.4%)	4 (16.7%)
No of vessels bypassed (mean)	2.73 (±0.96)	2.79 (±0.88)
Cross clamp time(min)	61.81 (±21.5)	57.25 (±22.7)
Cardiopulmonary Bypass time (CPB) (min)	92.6 (±31.6)	86.6 (±24.7)
Maintenance anaesthesia Isoflurane Sevoflurane	20 (76.9%) 8 (25.8%)	19 (79.2%) 5 (16.7%)
No of Grafts One Two Three More than three	3 (11.5%) 6 (23.1%) 13 (50%) 4 (15.4%)	3(12.5%) 3 (12.5%) 14(58.3%) 4 (16.7%)

4.7.1 Serum Troponin Release

Cardiac TnT levels were measured pre-operatively at the time of anaesthetic induction and at 6, 12, 24, 48, and 72 hours post cardiac surgery. The mean ($\pm$SD) cTnT levels at these time points is shown in table 4.9.3

Table 4.9.3

Time (Hours)	Control	RIPC	Mean difference	Confidence interval	P Value
6	0.61±0.40	0.58±0.45	0.03 ±0.12	-0.21 - 0.27	0.80
12	0.52±0.25	0.50±0.34	0.022±0.08	-0.14 - 0.19	0.79
24	0.41±0.22	0.38±0.22	0.025±0.06	-0.07 - 0.13	0.69
48	0.29±0.17	0.26±0.15	0.030±0.04	-0.10 - 0.15	0.51
72	0.26±0.18	0.24±0.16	0.016±0.04	-0.08 - 0.11	0.73

Values present mean ± SD (in µg/L)

There were no statistical differences in the cTnT levels between the RIPC group and the control group at 6, 12, 24, 48 or 72 hours. There was no significant difference in the cTnT AUC over 72 hours between two groups, mean cTnT AUC Conrol group 25.97 (±13.89) µg/L.72 hrs vs. 24.18(±14.44) µg/L.72 hrs in the RIPC group, p=0.65. There was no statistical difference in the maximum Troponin level over 72 hours between the two groups, maximum cTnT control group 0.66 (±0.37) µg/L vs. 0.62(±0.44) µg/L in the RIPC group, p = 0.70.

Fig 7: Troponin release post cardiac surgery

Error bars in the figure represent Std error of the mean
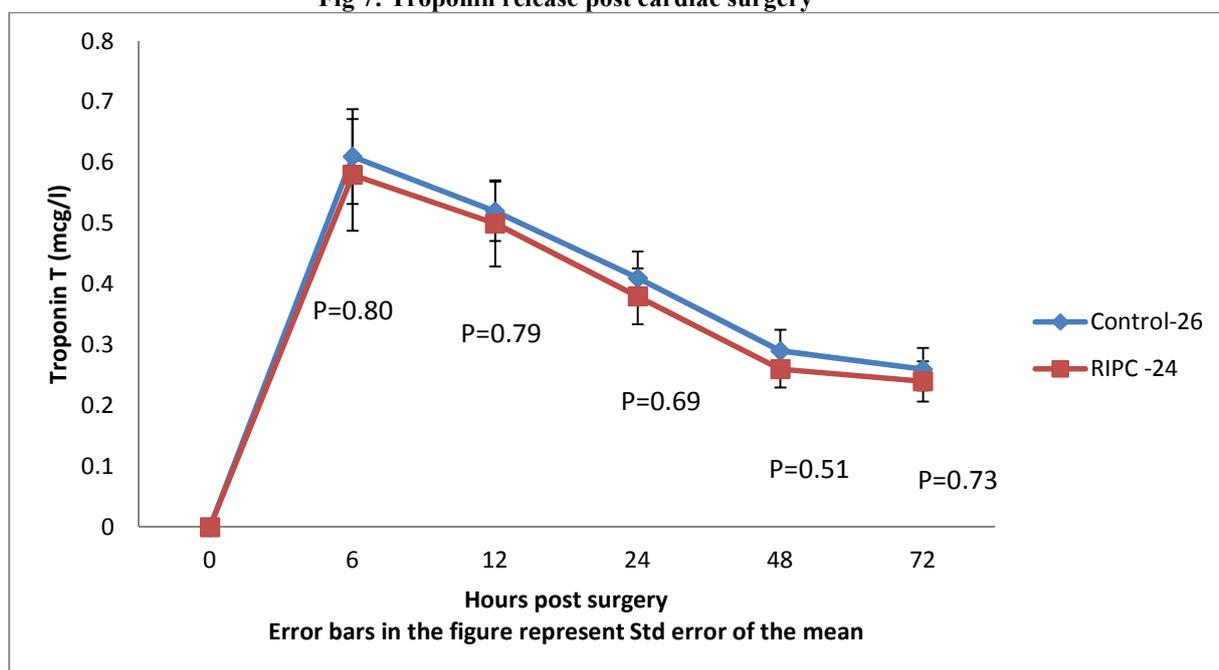
4.7.2 Serum CK-MB Release

Serum CK-MB mass were measured pre-operatively at the time of anaesthetic induction and at 6, 12, 24, 48, and 72 hours post cardiac surgery. The mean ($\pm$SD) CK-MB levels at these time points is shown in table 4.9.4.

Table 4.9.4

Time (Hours)	Control	RIPC	Mean difference	Confidence interval	P Value
0	3.54$\pm$0.72	3.87$\pm$2.3	-0.30$\pm$0.59	-0.15 - 0.89	0.61
6	25.37$\pm$12.8	23.77$\pm$10.1	1.60 $\pm$3.88	-6.30 – 9.51	0.68
12	20.68$\pm$10.3	18.23$\pm$7.3	2.44$\pm$2.96	-3.58- 8.47	0.41
24	17.83$\pm$10.1	15.31$\pm$6.9	2.52$\pm$2.86	-3.29 – 8.34	0.38
48	7.44$\pm$3.24	7.55$\pm$4.22	-0.11$\pm$1.30	-2.76 - 2.54	0.93
72	5.20$\pm$3.66	4.80$\pm$1.44	0.40$\pm$0.96	-1.56 – 2.36	0.68

Values present mean $\pm$ SD (in μg/L)

There were no significant differences in the CK-MB mass between the two groups at any time points postoperatively, i.e. at 0, 6, 12, 24, 48 or 72 hours. In addition, there was no statistical difference in the peak CK-MB mass levels over 72 hours between the two groups, peak CK-MB mass levels in control group 26.20 ($\pm$13.3) µg/L vs. 23.90 ($\pm$10.1) µg/L in RIPC group, p = 0.56.

4.7.3 Post-operative Ventilation Times

Post-operative ventilation times did not differ significantly between the two groups. Mean ventilation time for the control group was 7.74 ($\pm$5.80) hours vs. 8.32 ($\pm$3.84) hours in the RIPC group, p = 0.71.

4.7.4 Post-operative ITU Stay and High-care Stay

There were no significant differences in the duration of ITU stay between the two groups post-operatively. There was also no statistical difference in the duration of high-care stay between the two groups. Mean ITU stay (number of nights) in the control group 3.12 ($\pm$5.0) vs. 2.35($\pm$1.4) in the RIPC group, p= 0.51. Mean number of days of high care stay in the control group was 3.96 ($\pm$4.9) vs. 4.62 ($\pm$6.6) days in the RIPC group, p = 0.69.

4.7.5 Postoperative Inotrope Requirement and Inotrope Score

Data were obtained regarding the inotrope requirement post-operatively in all patients during their ITU stay. Based on the maximum inotrope concentration required, Inotrope-score was calculated using the formula: Inotrope score = Dosages (in µg/kg/min) of Dopamine + Dobutamine + [(Adrenaline + Noradrenaline + Isoproterenol) × 100] + [Enoximone × 15], adapted from Ko *et al*. (337).

Inotrope support was required in 5 (26.3%) of patients in the control group compared to 7 (35.0%) patients in the RIPC group. This difference was not statistically significant, p= 0.55. The inotrope scores among the patients requiring inotrope support did not differ significantly between the two groups. Inotrope score in the control group was 7.40 ($\pm$5.2) µg/kg/min vs. 12.18 ($\pm$10.3) µg/kg/min in the RIPC group, p = 0.36.

4.7.6 Post-operative Atrial Fibrillation (AF)

New AF occurred in 6 (23.1%) patients in the control group vs. 2 (8.3%) patients in the RIPC group. This was, however, not statistically different, p= 0.25.

4.7.7 Post-operative Acute Kidney Injury (AKI)

AKI was defined as an abrupt (within 48 hours) reduction in kidney function either with an absolute increase in serum creatinine of more than or equal to 0.3 mg/dl ($\geq$ 26.4 μmol/l), a percentage increase in serum creatinine of more than or equal to 50% (1.5-fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5 ml/kg per hour for more than six hours) (336).

AKI was observed in 4 (15.4%) patients in the control group vs. 2 (8.3%) patients in the RIPC group. This difference was not statistically significant, p = 0.66.

4.8 DISCUSSION

In this prospective open labelmrandomised controlled study involving Type 2 diabetic patients undergoing cardiac surgery, remote ischaemic preconditioning using 3 cycles of 5 minute upper limb ischaemia, did not demonstrate a significant reduction in the cardiac biomarker release (primary end point) postoperatively. There appeared no significant difference in the clinical endpoints of ventilation times, post-operative ITU stay, post-operative high care stay, post-operative AF incidence or post-operative AKI incidence between the two groups. These findings, did not change when a subgroup analysis of cardioplegic myocardial preservation cohort was analysed. I shall now discuss the possible reasons, why RIPC did not show any significant clinical or biochemical superiority in our study.

4.8.1 Possible Reasons of Lack of Superiority of Primary End Points in the **RIPC Group in** the **Diabetic Cohort.**

Three cycles of 5 minutes forearm ischaemia is a proven remote ischaemic stimulus that has shown clear reduction in Troponin AUC in non-diabetic patients undergoing CABG (270). The threshold of RIPC at which the myocardial protection manifests in the clinical setting is still unknown and it is possible that RIPC with two cycles of 5 minutes forearm ischaemia might also be protective in non-diabetic patients. Conversely, it might be that 3 cycles of 5 min ischaemia is the minimum ischaemic stimulus for any protection in the Troponin reduction to manifest. Most of the cardiovascular clinical studies involving RIPC have used 3 cycles of 5 min remote ischaemic stimulus in their studies(78, 79, 163, 270, 271, 338). In our Institute we are currently investigating if lower or higher ischaemic stimuli are as protective as 3 cycles of RIPC stimulus.

From the animal studies we do know that, in Type 2 diabetic rats, preconditioning with standard ischaemia stimulus fails to demonstrate the reduction in infarction, and that a prolonged ischaemic stimulus is needed to precondition diabetic hearts in these animal models (318, 324). Similar observations were shown in the human atrial trabeculae model by Sivaraman *et al.* (325), where a standard preconditioning protocol failed to demonstrate

contractile recovery in diabetic human trabeculae subjected to 90 min ischaemia. By prolonging the ischaemic preconditioning stimulus, the contractile recovery similar to non diabetic myocardium was demonstrated in diabetic human trabeculae. When translating this effect clinically and evaluating remote ischaemic stimulus in cardiac surgery, it is not surprising that a standard stimulus of 3 cycles of 5 minute forearm ischaemia might not have sufficient preconditioning stimulus in diabetic patients, particularly, if 3 cycles of 5 minute ischaemia is the threshold stimulus in non-diabetic patients. Certainly, the only way to evaluate this hypothesis is by investigating whether a prolonged remote ischaemic preconditioning stimulus reduces the perioperative Troponin release in Type 2 diabetic patients. In addition, characterisation of RIPC in non-diabetic patients with 2 cycles of 5 minute ischaemia and reperfusion, and even 1 cycle of 5 minute ischaemia and reperfusion will likely establish the threshold remote ischaemic stimulus in clinical patients for reducing the myocardial injury during cardiac surgery.

Secondly, in the animal studies, the ischaemic standard stimulus has been at least 90 minutes or over, and this is an absolute ischaemia. In clinical studies, the ischaemic stimulus (cross clamp time) in most cardiac surgeries has been around 60 minutes. In addition, this is not absolute ischaemia as the myocardium itself is perfused with cardioplegia solution, which reduces the ischaemic effect significantly. Importantly, if the surgical cardioprotection method is cross clamp fibrillation rather than cardioplegic cardioprotection, then this could be considered as absolute ischaemia. However, cross clamp fibrillation method will only involve maximum period of 20 minutes and, therefore, it never renders the ischaemic burden similar to the animal model settings. It is important to recognise that the infarct size seen in animal models are never seen in human cardiac surgeries. Consequently, the clinical endpoints in most clinical studies are surrogate markers like Troponins. It could, therefore, mean that the clinical translation for evaluation of preconditioning is being conducted in significantly different conditions of ischaemia-reperfusion settings, i.e. smaller, altered settings of myocardial ischaemia, and confounded by the presence of other preconditioning agents, which will be discussed later. This is particularly relevant in diabetic patients

undergoing cardiac surgery, as we know from the animal models that, in particular conditions, diabetic hearts are relatively less susceptible to ischaemic insult than non-diabetic myocardium. In our study, diabetic patients in the control group had a 72 hour Troponin area under the curve of 24.55 µg/L.72hrs. This is much smaller than the control group 72 hour Troponin AUC in the previous study from our own Institute, evaluating RIPC in the non-diabetic patient group in similar settings, and in the same surgical centre, with a value of 31.53 µg/L.72hrs (270) Certainly, this observation strengthens the hypothesis that human diabetic myocardium is resistant to index ischaemic insult, translating from the similar findings in the animal models.

The use of inhalational anaesthetics, which do provide preconditioning effect with some of the intrinsic mechanisms similar to ischaemic preconditioning, is well known. Anaesthetic preconditioning is discussed in detail in the previous chapter (see section 1.13). Most of the patients undergoing cardiac surgeries are subjected to these volatile anaesthetics. Most of our patients in this study had Isoflurane (75%) or Sevoflurane (25%) as maintenance anaesthesia during the cardiac surgery. Certainly, this is an important confounding factor seldom experienced in animal models. Exposure to anaesthetic preconditioning could have diluted the effect of remote ischaemic preconditioning in our patient cohort, which partly explains similar AUC in both the control group and the RIPC group. However, our Institute's previous studies on non-diabetic CABG patients evaluating RIPC, was performed under similar settings and similar anaesthetic protocols, and those studies have shown significantly improved troponin levels in the RIPC group (79, 270). This suggests that RIPC could provide additional protection over and above of that provided by volatile anaesthetics. However, two recent studies have failed to demonstrate any clinical or biochemical superiority of RIPC in the settings of elective CABG. These include a large double blind randomised study involving 162 patients undergoing elective CABG with standard blood –cardioplegia by Bonser *et al.* (272), and another smaller study involving 54 patients undergoing elective CABG with either cardioplegia or cross–clamp fibrillation as the myocardial preservation strategy, by Kunst *et al.*(273). Both these studies included a similar RIPC stimulus, and were conducted in patients undergoing coronary artery bypass surgery. The study by Bonser *et al.* (272), excluded patients with diabetes mellitus, and all patients had cardioplegia as surgical cardioprotection

method. The study by Kunst *et al.* (273), however, included patients with diabetes mellitus, and had patients undergoing surgery with either cardioplegia or intermittent cross clamp fibrillation as the surgical cardioprotection method. The findings of both these studies have raised doubts if RIPC adds to any further significant cardioprotection over and above that provided by inhalational anaesthetics. A recent study by Lucchinetti *et al.* specifically studying the effect of remote preconditioning in Isoflurane anaesthetised patients during CABG, showed no added benefit from preconditioning(339). In another recent study, looking at remote preconditioning in valvular heart surgery has reported 44% reduction in Troponin I area under the curve over 72 hours (340). Maintenance anaesthesia was predominantly with sufentanil in these patients, and this cohort included diabetic patients.

Interestingly, anaesthetic preconditioning effect in diabetic animal models appears to be significantly less compared to non-diabetic models (288). This appears to be due to similar protective pathways involved in anaesthetic preconditioning and in ischaemic preconditioning. For this reason, confounding effect of volatile anaesthetics could be thought to be not so important in a diabetic population. In our study, there was no difference in the proportion of patients in both the control and RIPC groups receiving volatile aneasthetics as maintenance anaesthesia.

Cardio pulmonary bypass (CPB), itself is thought to provide preconditioning stimulus and, therefore, RIPC might not have added further cardioprotection. In a study by Ghosh *et al.* (341), who evaluated classic ischaemic preconditioning in the reduction of post-operative troponin levels, found that IPC did not affect Troponin release in patients undergoing CABG with either cardioplegia or cross-clamp fibrillation. IPC however reduced Troponin levels significantly in patients undergoing CABG on the beating heart (without the use of CPB). Although exact mechanisms are unclear, it is thought that systemic inflammatory response and altered adrenergic state conferred by CPB, contribute to the preconditioning effect (341).

Therefore, there appears to be a few reasons why RIPC in our clinical study has failed to demonstrate reduction in cardiac enzyme levels, when compared to the control group. Patients undergoing CABG are a very heterogenous group. The duration of CPB and cross

clamp times, volume of inhalational anaesthetics required, relative myocardium at risk, which is exposed to ischaemia during surgery, use of drugs with inherent preconditioning effect, and presence of angina prior to CABG are very different in each individual and, clearly, cardiac enzyme release do vary significantly among different patients. Smaller studies like ours might not be able to definitely answer the research question, whether RIPC is a further myocardial protective agent or not in this context, and consequently larger multicentre studies are required to answer this important question. ERICCA is a large multi-centre randomised trial which is currently underway involving 1610 patients and is powered for clinical outcomes(342). This study is likely to answer and clarify the prevailing questions and doubts about remote ischaemic preconditioning during CABG.

5. SUMMARY, CONCLUSIONS AND FUTURE DIRECTIONS

Remote Ischaemic Preconditioning is a simple non-invasive effective cardioprotective method, which has a role in various clinical contexts. Our two clinical trials involved evaluation of RIPC in two specific groups of population i.e. NSTEACS patients undergoing urgent in-hospital PCI, and in Type 2 diabetic patients undergoing elective cardiac bypass surgery. RIPC in both these trials did not show significant clinical or biochemical superiority compared to standard (control) therapy. The possible reasons of this lack of significant difference in primary end points have been discussed earlier. Our studies reinforce the need for alternative or more intensive cardioprotective strategies in these high risk groups undergoing myocardial revascularisation.

Our studies raise the research question of 'what is the threshold remote preconditioning needed for clinical cardioprotection?' Consequently further clinical studies evaluating and characterising remote preconditioning were proposed by our Institute and with ethical approval, these studies are already in progress. In Type 2 diabetic patients, the role of increased and prolonged remote preconditioning stimulus has now been investigated in our Institute, involving patients undergoing cardiac surgery as a direct result of the current findings. In addition, with the improved peri-operative and per-operative surgical techniques, reducing cross clamp times, ever improving anaesthetic agents with inherent cardioprotective properties, and with concomitant routine use of cardioprotective drugs in routine cardiac surgery (e.g. Nitrates, Statins), the role of further cardioprotection through interventions like RIPC might need careful selection of patients. This is likely to be beneficial in patients who would be predicted to have prolonged cross clamp times during surgery, as the ischaemic burden is higher. This is usually the case in patients undergoing surgical coronary revascularisation along with valve surgeries/repairs. In these surgical procedures, relatively more myocardium would be theoretically salvageable through measures like RIPC over and above the standard treatment, as the ischaemic burden is much higher due to prolonged cross clamp times. A separate study, involving complex cardiac surgeries with prolonged cross clamp times, and evaluating the effect of RIPC has also been currently evaluated at our research centre.

The results of our studies do not question RIPC as a cardioprotective agent, but only emphasise that RIPC may not be significantly beneficial in certain clinical groups and clinical situations. It should also be appreciated that most of the previous studies involving RIPC, excluded the diabetic population from their studies, particularly as animal models did not show protective effect in IR situations. Our RIPC study in diabetic CABG patients translates with the similar results in the human clinical situation. Similarly in the PCI settings, patients with recent ACS are known to have increased peri-procedural myocardial injury due to unstable soft friable plaques. RIPC in this setting, unfortunately, did not show a clear trend in reduction of peri-procedural myocardial enzyme release. The possible reasons were discussed in the previous chapter. Ironically, the two groups studied in our studies are the patients at much higher risk, and who were in most need for further cardioprotection. The findings only suggest that a much stronger cardioprotection strategy either on its own or via a combination of available cardioprotection strategies (pharmacological and mechanical), must be evaluated in these resistant groups of patients who are in most need of such cardioprotection. In addition, the role of combination of remote preconditioning and post conditioning in these settings is a potential strategy to explore. More importantly, large multicentre randomised controlled trials with hard clinical end points would answer and clarify many unanswered questions of effective cardioprotection of remote preconditioning in clinical practice of cardiac surgery.

A summary of all the studies which have used remote ischaemic preconditioning in various cardiac surgery settings and in other cardiac interventions are summarised below.

Table.5.1

Cardiac Surgery

Study	No of Patients	RIC Stimulus	Patient Group	Outcomes
Cheung(78)	37	Lower limb (4 X 5 min)	Elective pediatric cardiac surgery	↓cTnI (P ¼ .04) ↓ Inotrope score (7.0 vs 10.9 mcg/kg/min) ↓ Airway resistance (34 vs 49 cmH2O/mL/s)
Hausenloy(79)	57	Upper limb ischaemia (3 X 5 min)	Elective CABG ± valve surgery(cold blood cardioplegia or cross-clamp fibrillation)	↓AUC of cTnT (43%)
Venugopal(270)	45	Upper limb ischaemia (3 X 5 min)	Elective CABG ± valve surgery(cold blood cardioplegia only)	↓AUC of cTnT (42.4%)
Ali (343)	100	Upper limb ischaemia (3 X 5 min)	Elective CABG	CK-MB (mean reduction 3 IU/L)
Thielmann (271)	53	Upper limb ischaemia (3 X 5 min)	Elective CABG (Cold crystalloid cardioplegia)	↓AUC of cTnI (44.5%)
Hong (344)	130	Upper limb ischaemia (4 X 5 min)	Elective CABG (off-pump)	Nonsignificant 26% reduction in cTnI release
Rahman (272)	162	Upper limb ischaemia (3 X 5 min)	Elective and urgent CABG	No difference in cTnT, ECG changes, inotrope score, renal and lung injury
Li (345)	81	Lower limb ischaemia (3 X 4 min)	Elective valve replacement	↓cTnI peak level (40%) but no difference in total AUC in the perconditioning group compared to control
Zhou (346)	60	Lower limb ischaemia (3 X 5 min)	Elective pediatric cardiac surgery	↓Inotrope score (12.0 vs 15.87 mcg/kg/min at 4 hours; 8.63 vs 10.67 at 12 hours) ↓LDH/CK-MB/cTnI ↓Lung injury

Lucchinetti (339)	55	Lower limb ischaemia (4 x 5 min)	Elective CABG surgery with Isoflurane as maintenance anaesthesia	No difference in hs-troponin T release compared to placebo.
Xie JJ (340)	83	Upper limb ischaemia (3 X 5 min)	Elective heart valve surgery	↓AUC of cTnT (43.2%)

Non Cardiac surgery

Ali (80)	82	Lower limb ischemia (2 X10 min)	Elective AAA Repair	↓ AUC of cTnI (27%) ↓ Perioperative MI (22%) Preserved renal function
Walsh(347)	40	Sequential lower limb ischemia (1 X 10 min on each limb)	Elective EVAR	No difference in rates of renal outcome indices and cardiac events
Walsh (348)	40	Sequential lower limb ischemia (1 X 10 min on each limb)	Elective open infrarenal AAA repair	No difference in rates of renal outcome indices
Walsh (349)	70	Sequential lower limb ischemia (1 X 10 min on each limb)	Elective carotid endarterectomy	No difference in cardiac and neurological outcomes

Elective PCI

Iliodromitis (269)	41	Bilateral upper limb ischemia (3 X 5 min)	Elective PCI	↓ CRP levels at 49 h (1.7 mg/L difference) ↓ CK-MB (62% AUC) ↓ cTnI (16% AUC)
Hoole (268)	202	Upper limb ischemia (3 X 5 min)	Elective PCI	↓ Median cTnI concentration at 24 h (0.06 vs 0.16 ng/mL) ↓ MACCE (HR 28%) ↓ CP/ST changes
Hoole (350)	54	Upper limb ischemia (3 X 5 min) or TV	Elective PCI	No difference in microvascular resistance or coronary flow velocity

		balloon occlusion		
Hoole (351)	42	Upper limb ischemia (3 X 5 min)	Elective PCI	No improvement of ischemic LV dysfunction and stunning
Prasad (352)	95	Upper limb ischemia (3 X 5 min)	Elective and Unstable angina PCI	No difference in the incidence of periprocedural myocardial necrosis

Primary PCI

Rentoukas (353)	96	Upper limb ischemia (4 X 4 min, 20 mmHg above SBP)	Primary PCI (STEMI)	Improved ST segment resolution (82% RIC +Morphine, 73% RIC, 53% control) ↓Peak cTnI (RIC + Morphine: 103.3 ng/mL, morphine alone RIC: 166 ng/mL, control: 255.5)
Botker(338)		Upper limb ischemia (4 X 5 min)	Primary PCI (STEMI)	↓ Myocardial salvage at 1 month (median difference of median salvage index 0.12 and mean difference of mean salvage index 0.12) No difference in cTnT release No difference in MACCE

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