

**Effect of Remote Ischaemic  
Preconditioning in patients undergoing  
complex PCI  
(The ERIC-PCI trial)**

**By**

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A thesis presented on the cardioprotective effect of Remote Ischaemic Preconditioning in complex PCI setting.

# Declaration

I, Shaqayeq (Shana) Tehrani, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, this has been indicated.

Signed

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# ABBREVIATIONS and ACRONYMS

AAR=area-at-risk	MPTP= mitochondrial permeability transition pore
ACE-I=angiotensin converting enzyme inhibitor	MSI=myocardial salvage index-
ACS=acute coronary syndrome	NGAL= neutrophil gelatinase associated lipocalin
ARB=angiotensin II receptor blocker	PCI=percutaneous coronary intervention
BMS=bare metal stent	PKC=protein kinase c
cTn=cardiac troponin	PPCI=primary PCI
CABG=coronary artery bypass graft	R&D=research and development
CGRP=calcitonin gene related peptide	RIPC=remote ischaemic preconditioning
CHD=coronary heart disease	RISK=reperfusion injury salvage kinase
CI-AKI=contrast-induced acute kidney injury	ROS=reactive oxygen species
CK-MB=creatinine-kinase MB	RWMA=regional wall motion abnormality
CMR= cardiac magnetic resonance	STEMI=ST elevation MI
CTO=chronic total occlusion	SV=stroke volume
CVA=cerebrovascular accident	SVG=saphenous vein graft
CVD=cardiovascular disease	SWOP=second window of preconditioning
DES= drug eluting stent	TIMI=thrombolysis in myocardial infarction
ECG=electrocardiogram	VF=ventricular fibrillation
eGFR=estimated glomerular filtration rate	VT=ventricular tachycardia
GP IIb/IIIa =glycoprotein IIb/IIIa	
GPCR=G protein-coupled receptors	
GTN=glyceryl trinitrate	
hsTnT=high sensitive troponin T	
IPOC=ischaemic postconditioning	
ISDN= isosorbide dinitrate	
LGE=late gadolinium enhancement	
LV=left ventricle	
LVEDV= left ventricular end diastolic volume	
LVESV=left ventricular end systolic volume	
MI=myocardial infarction	

# ABSTRACT

**Background-** Coronary Heart Disease (CHD) is one of the leading causes of death and disability worldwide. Revascularisation of the coronary arteries using Percutaneous Coronary Intervention (PCI) has become the treatment of choice for most of the patients with CHD. Despite significant advances in angioplasty technique and introduction of anti-proliferative medications in drug eluting stents, myocardial injury during PCI is still significant. In this thesis we hypothesised whether Remote Ischaemic Preconditioning (RIPC) can reduce PCI-related myocardial injury.

**Methods-** Eighty-eight patients awaiting elective complex PCI were randomly assigned in a 1:1 ratio to receive either the RIPC (intermittent arm ischaemia and reperfusion through four cycles of 5-minutes inflation and 5-minutes deflation of a blood-pressure cuff placed on the upper arm) or control (un-inflated cuff placed on upper-arm for 40 minutes) prior to PCI. The primary endpoint was reduction of the incidence and extent of PCI-related myocardial injury, assessed by serum cardiac biomarkers, 24 hours post PCI.

**Results-** The Troponin T level at 24 hours post PCI was 48 ng/l in the control group vs 32.5 ng/l in the RIPC group,  $P = 0.39$ . There was no significant reduction in the total area under the curve (AUC) in the RIPC group,  $P = 0.43$ . Regarding the incidence of PCI-related myocardial injury, significant elevation of Troponin level post PCI ( $> 5 \times$  baseline), was observed in 46.9% of patients in the control group and in 26.7 % of patients in the RIPC group,  $p = 0.12$ .

**Conclusion-** In the ERIC-PCI study, RIPC did not demonstrate a statistically significant attenuation of serum Troponin release post PCI. The results however showed a positive trend towards efficacy of RIPC in reducing PCI-related myocardial injury.

# **CHAPTER 1**

## **INTRODUCTION**

Cardiovascular disease (CVD) is set to remain the leading cause of death worldwide with the death toll rising to 23 million annually by 2030. The World Health Organisation (WHO) has reported that an estimated 17 million people die of CVD each year. Of these deaths, 80% due to coronary heart disease (CHD).<sup>(1)</sup>

In 2013 there were 1.9 million deaths from cardiovascular disease in EU, which was equivalent to 37.5 % of all deaths — considerably higher than the second most prevalent cause of death, cancer; 26.0 %.<sup>(1)</sup>

In the United States, cardiovascular disease including CHD and stroke, accounts for more than one-third of deaths with an estimated 900,000 heart attacks and 800,000 strokes occurring each year. A similar pattern is seen in the rest of the world, from Asia to Africa and to Europe.<sup>(2)</sup>

Revascularisation of the coronary arteries using percutaneous coronary intervention (PCI) has become the treatment of choice for most patients with CHD, with a rapidly growing prevalence over the last few years. In Europe, 1.5 million people had PCI in 2010; over 87,000 of these procedures were conducted in the UK. In the United States, approximately 1.5 million patients undergo PCI every year.<sup>(2)</sup>

Increased life expectancy and subsequent changes in patients' demographics have resulted in the increased prevalence and complexity of coronary artery disease in the elderly patients.

PCI however only improves symptoms and has no positive impact on the prognosis. In fact, injury of the myocardium secondary to various insults caused by PCI is a recognised complication (PCI-related myocardial injury or type 4 a MI).<sup>(3)</sup> Despite innovations in the field of interventional cardiology, introduction of novel medications, and advances in operator skills, myocardial injury during PCI is still significant. Depending on local practice and the diagnostic criteria used, 5 to 30% of the 1.5 million patients who undergo planned PCI every year (75,000 to 450,000), have evidence of PCI-related myocardial injury.<sup>(4-8)</sup> PCI-related myocardial necrosis or injury, even

with no immediate clinical presentation, is accompanied by the release of structural proteins and intracellular molecules into the cardiac interstitium and blood-stream. Therefore, this form of PCI-related myocardial injury, which is often clinically silent, can be detected as an increase in serum cardiac enzymes above the 99th centile upper reference limit. Release of Troponin and CK-MB has a poor impact on prognosis of patients with CHD.<sup>(9-12)</sup> The amount of enzymes released at the time of PCI, correlates with subsequent infarct size and prognosis.<sup>(13)</sup>

A post-procedural increase in cardiac troponin (cTn) concentration of  $\geq 5$  fold baseline levels is an independent predictor of composite of death, myocardial infarction, and revascularisation at 1 year [HR, 2.39; 95% CI, 1.09 to 5.26].<sup>(13)</sup> Thus, the pathogenesis of PCI induced myocardial injury and how to prevent this damage has been the subject of extensive research.

Myocardial injury sustained during PCI is the result of distal and peri-stent microcirculatory obstruction, distal coronary artery embolisation, side-branch occlusion, coronary dissection, occlusion of collaterals and epicardial or microvascular spasm. Although not entirely agreed by all scientists, cardiomyocyte damage with troponin release may also be associated with a form of ischaemia/reperfusion injury during stent deployment and sudden supply of blood to the ischaemic tissue<sup>(14, 15)</sup>

Some major risk factors which increase susceptibility to procedural related myocardial injury during PCI include age, diabetes, multi-vessel coronary artery disease (CAD), diffuse CAD, systemic atherosclerosis, pre-existing renal impairment, bleeding tendency and the presence of anaemia.

The concept of myocardial injury during or following angioplasty has drawn the attention of scientists over the last few years with much research taking place worldwide to understand the pathophysiology behind this form of injury (termed peri-procedural myocardial injury or PMI), and to find the potential approach to cardioprotection. Since PMI is associated with worse

clinical outcomes after PCI, reducing the incidence and magnitude of PMI during planned PCI procedures may reduce major adverse events.

Therapeutic options available for reducing PMI have been mainly limited to improvements in antiplatelet, antithrombotic and statin therapy as well advances in PCI technology and distal protection devices. In the TRITON-TIMI 38 Trial comprising 13608 patients undergoing PCI, Prasugrel reduced the risk of PMI by 24% in comparison with clopidogrel. <sup>(16)</sup>

Despite above advances in medications, devices and operators skills, peri-PCI injury or infarction is still common. Reducing myocardial injury during PCI will significantly improve the outcome and will reduce the MACE. Interventions that reduce this injury have been the subject of research in recent years.

Thus, there is a potential role for adjunctive methods such as preconditioning, which may provide cardioprotection during PCI and render the heart resistant against ischaemia and reperfusion injury and complications of PCI. Although due to reperfusion injury concept, the focus has been most on reducing injury during PPCI, there has also been interest in utilising RIPC to attenuate peri-procedural MI in patients undergoing non-emergent elective PCI.

There are a number of ways in which preconditioning can be induced. Details of different types of preconditioning are mentioned in next pages of this thesis but in summary: A) 'Local preconditioning' occurs when the preconditioning stimulus is applied to the same organ or tissue that will subsequently sustain the ischaemic injury. B) 'Remote ischaemic preconditioning' which refers to a stimulus applied to a distant organ or tissue, and then protects against index ischaemia. C) 'Postconditioning' occurs when there is staged reperfusion, for example, in the setting of balloon angioplasty. Its variant D) 'per-conditioning' occurs when the conditioning stimulus is applied during ischaemia.

Remote Ischaemic Preconditioning (RIPC) is a major breakthrough in clinical applicability of preconditioning method due to being safe, easy to perform and cost free. Although RIPC is one of the most powerful and reproducible phenomena in cardioprotection with promising experimental data, it has not been readily translated into the routine clinical practice because of methodological obstacles. The outcome of the clinical findings also has been conflicting with inconsistent results. Therefore, at present there is no preconditioning-based therapy that is routinely used in clinical practice. Nonetheless, despite the consensus that, in experimental models, ischaemic conditioning has a profound infarct sparing effect, 'the outcome of attempting to translate this most potent and basic cardioprotective response to the clinical environment has been described as frustrating and disappointing.

Myocardial injury related to PCI is reportedly higher in procedures which are so called 'complex', with higher possibility of PCI-related myocardial injury compared with simple single vessel type A lesions. Following Hoole's trial in 2009<sup>(15)</sup>, which showed significant cardioprotective efficacy of RIPC in elective PCI, a similar proof of concept study was performed by The Hatter Institute in 2011 which failed to confirm similar positive findings. (*Babu et al .Unpublished study*) Therefore we at the Hatter Institute hypothesised that RIPC might reduce peri-PCI myocardial injury in complex PCI. Complex PCI is generally applied to the procedures with challenging anatomy i.e. SYNTAX score  $\geq 23$ , severely calcified coronary arteries requiring rotational atherectomy, chronically occluded arteries (CTO), or stenosis in saphenous vein grafts (SVG) which might require laser assisted PCI. Therefore in the randomised-controlled ERIC-PCI trial, we hypothesised that the safe and low-cost therapeutic intervention of RIPC which refers to a powerful endogenous protective phenomenon by applying brief episodes of non-lethal

ischaemia and reperfusion to the upper limb will reduce the peri-complex PCI complications as evidenced by less Troponin release in the blood and less LGE in the CMR.

As preconditioning is a systemic response, and preconditioning applied to one organ confers protection against a sustained lethal episode of ischaemia and reperfusion in another organ, we hypothesised that RIPC is a potential strategy for preventing CI-AKI in our trial.



## PCI-related Myocardial Injury

PCI has become the revascularisation method of choice for most of the patients with CHD. Despite recent transformations in the safety of this procedure through new medicine and device innovations, complications during PCI are still common and significant. This leads to a worse outcome even after a successful revascularisation. In some of these cases, complications can be clinically evident, but evidence of myocardial injury can also be detected after routine uneventful PCI procedures.

After PCI, a reduced coronary flow velocity reserve (CFVR) is often observed.<sup>(17)</sup> This is associated with an increase in cardiac enzymes. During PCI, levels of Creatine Kinase (CK) or CK-MB isoenzyme can increase in 3–30% of patients.<sup>(18-23)</sup> Levels of high sensitive cardiac Troponin (I or T) are elevated to even greater levels, often in 30–40% of elective cases.<sup>(4-6)</sup> Procedure-related cardiac enzyme release is associated with subsequent cardiovascular events which make the outcome of a visually successful intervention unfavourable. Several studies have confirmed the relation between higher cardiac enzyme release post PCI and worse prognosis.<sup>(9, 13, 24-27)</sup> Surprisingly, controversy still exists about the clinical significance of procedural events during PCI.<sup>(8, 28)</sup>

There are uncertainties about the pathogenesis and mechanism of small cardiac enzyme elevations following PCI. Microembolisation of plaque debris in the distal vasculature, inflammation causing microvascular obstruction, oedema, slow flow or no-flow and side-branch occlusion have been proposed as the most likely mechanisms of cardiac troponin release.<sup>(3, 15, 20, 29)</sup> Major vessel occlusion, coronary dissection and thrombus formation, result in more significant troponin release.

Microembolisation might not be clinically apparent in the catheterisation lab. It may be entirely asymptomatic and occur even during or after angiographically uneventful procedures and be visualised by cardiac MRI. <sup>(20, 23, 29, 30)</sup>

An intra-coronary Doppler flow-wire study has been used by Bahrmann et al. to evaluate the incidence of coronary microembolisation during coronary interventions.<sup>(17)</sup> This method was previously established to detect emboli in cerebrovascular flow. Investigations by Bahrmann and his colleagues showed that the number of microembolic high-intensity signals (HITS) during PCI correlated with cTnI release.<sup>(17)</sup> The highest frequency of high-intensity signals is seen during stent deployment, although the background signal is increased throughout PCI. The number of HITS after stent deployment is significantly higher than just balloon angioplasty. This can be explained by the different mechanism of balloon dilatation and stent insertion. Balloon angioplasty enlarges the lumen by local dissection, whereas stenting works through plaque compression.<sup>(31)</sup>

New devices, which provide distal protection during transcatheter treatment of saphenous vein graft stenosis (SVG), have supported the concept of coronary microembolisation. <sup>(32, 33)</sup>

Thrombotic (platelet) and non-thrombotic materials ranging in size from 100–550 μm including cholesterol crystals, fibrin and lipid rich macrophages were detected in aorto-coronary SVGs as well as in native coronary arteries.<sup>(34, 35)</sup> The dominant role of platelets in this phenomenon is supported by the evidence of marked decrease in CK-MB elevation, achieved with administration of potent antiplatelet agents such as glycoprotein IIb/IIIa inhibitors. <sup>(30, 36)</sup>

The distal embolic debris contribute to the development of no-reflow and myocardial injury.

Cardiac Magnetic Resonance (CMR) scan with late gadolinium enhancement has demonstrated that procedural cTnI release is mostly due to myocardial necrosis resulting from distal embolisation of particular materials during balloon inflation and stenting.<sup>(37)</sup> It was also shown

by Selvanayagam et al. that new hyperenhancement post-PCI can be located in the basal or mid-ventricular myocardium, adjacent to the inserted stent.<sup>(37)</sup> This finding might suggest a side-branch flow impairment/occlusion. Although the magnetic resonance imaging studies suggest the myocardial injury sustained during PCI is the result of more distal and peri-stent microcirculatory obstruction and/or side-branch occlusion, cardiomyocyte damage and troponin release also may be associated with a form of ischaemia/reperfusion injury during stent deployment.<sup>(14, 15)</sup>

The concept of coronary no-flow will be discussed in details later in this chapter.

There are a few studies that have confirmed the pathogenesis of CK-MB elevation based on the interventional procedures performed. Mehran et al. explained the direct relationship between atherosclerotic plaque burden and cardiac enzyme release with the aid of intra vascular ultrasound (IVUS) prior to the procedure<sup>(38)</sup> Greater lesion and reference segment plaque burden, severe calcium, and positive remodelling were all associated with CK-MB elevation. Lesion-associated coronary artery calcium increases with extent and severity of atherosclerosis and correlates with volume of the atherosclerotic plaque.<sup>(39)</sup>

Several other parameters, including more aggressive intervention, balloon size, stent length and acute gain in vessel diameter, have also been shown to be related to PCI-associated cTnI release.<sup>(20, 23, 24, 37, 38, 40, 41)</sup> Drug-eluting stent (DES) implantation is associated with a higher incidence of procedural-related elevation of cTnI compared with bare metal stent (BMS) implantation.<sup>(11, 42)</sup> Bifurcation lesions or diffuse lesions are not necessarily positive predictors of peri-procedural injury.

## **Universal Definition of PCI-related Myocardial/Injury Infarction (Type 4a MI)**

In 2007 the ESC/ACC/AHA/WHO proposed a definition for PCI-related injury.<sup>(43)</sup> This definition has been updated since its introduction with the third version released in 2012.<sup>(3)</sup> According to the latest guidelines from the European Society of Cardiology/American College of Cardiology/American Heart Association/World Health Federation (ESC/ACC/AHA/WHO),<sup>(3)</sup> in patients undergoing PCI with prior normal (<99th percentile URL baseline cardiac troponin (cTn) concentrations, elevations of cTn >5 x 99th percentile upper reference limit (URL) occurring within 48 hours of the procedure—plus either (i) evidence of prolonged ischaemia (>20 min) as demonstrated by prolonged chest pain, or (ii) ischaemic ST changes or new pathological Q waves, or (iii) angiographic evidence of a flow limiting complication, such as of loss of patency of a side branch, persistent slow-flow or no-reflow, embolisation, or (iv) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality—should be defined as PCI-related MI (type 4a).

The threshold of cTn values >5 x 99th percentile URL was arbitrarily chosen, and was based on clinical judgment and societal implications of the label of procedural-related MI. When a cTn value is < 5 x 99th percentile URL after PCI with a normal value before the PCI—or when the cTn value is >5 x 99th percentile URL in the absence of ischaemic, angiographic or imaging findings—the term ‘PCI-related myocardial injury’ should be used rather than ‘myocardial infarction’. Other diagnostic criteria for PCI-related MI include an elevation of creatine kinase-MB (CK-MB) fraction >3x the upper limit of normal (ULN), which is supported by studies correlating CK-MB increase post-PCI with subsequent risk for ischaemic events.<sup>(38, 44-46)</sup>

## Cardiac Biomarkers and Diagnosing Peri-PCI Myocardial I-njury

Peri PCI myocardial injury or necrosis results from complications of mechanical revascularisation of the coronary arteries, including distal embolisation, side branch occlusion, coronary dissection, slow flow, no-reflow or even perhaps reperfusion injury. Despite current anticoagulant and antiplatelet adjunctive therapy and aspiration or protection devices, embolisation of intracoronary thrombus or atherosclerotic debris may not be preventable. Such events induce inflammation of the myocardium and possible subsequent necrosis. PMI can be clinically evident or silent, angiographically visible or invisible. The laboratory diagnosis of myocardial necrosis depends on elevation of sensitive and specific serum cardiac biomarkers such as cardiac Troponin (cTn) or the MB fraction of creatine kinase (CK-MB).

The cTn complex consists of three subunits: Troponin T, I and C. Cardiac troponin I and T are regulatory proteins of contractile apparatus of myocardial cells that control the calcium mediated interaction between actin and myosin. Cardiac troponin T (cTnT) binds tropomyosin and facilitates contraction; cardiac troponin I (cTnI) binds actin and inhibits actin myosin interactions; and troponin C (TnC) binds calcium ions. TnC is not used clinically because both cardiac and smooth muscle share a common isoform.<sup>(47)</sup>

The elevations of these biomarkers in the blood reflect injury leading to necrosis of myocardial cells and can be detected by measurement of cardiac biomarkers before the procedure, repeated 3–6 hours later and, optionally, further re-measured 12-24 hours later. Current commercial assays endeavour to use a population defined upper limit of normality at the 99th percentile as recommended by the guidelines. This is the level at which 99 out of 100 people in a healthy population will have a negative result (one in 100 will have a false positive result).<sup>(47)</sup> In a meta-analysis of 15581 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. <sup>(48)</sup> The underlying mechanism of Troponin release however is not entirely clear. Various explanations have been suggested for the release of structural proteins from the myocardium, including normal turnover of myocardial cells, apoptosis, cellular release of troponin degradation products, increased cellular wall permeability, formation and release of membranous blebs, and myocyte necrosis. <sup>(49)</sup>

The recent development of high sensitivity Troponin (hsTn) assays in 2011, has improved sensitivity for the detection of myocardial necrosis compared with conventional assays. In the evaluation of patients with suspected MI, use of hsTn assays provides superior diagnostic accuracy with very high sensitivity and negative predictive value compared with conventional assays. The increased sensitivity however results in measuring values of hsTn in the majority of normal subjects. Therefore, following debates about the significance of elevation of Troponin and epidemiological/psychosocial consequences of being diagnosed with MI, in 2012, the Study Group on Biomarkers in Cardiology of ESC redefined the Peri-PCI MI and increased the arbitrarily chosen threshold for Troponin release from x 3 times URL to x 5 times URL. Recognising the need for a universal definition for peri-PCI MI or Type 4a MI, it was defined as elevations of cTn x 5 99th percentile URL occurring within 48 hour of the procedure— plus either (i) evidence of prolonged ischaemia (20 min) as demonstrated by prolonged chest pain, or (ii) ischaemic ST changes or new pathological Q waves, or (iii) angiographic evidence of a flow limiting complication, such as loss of patency of a side branch, persistent slow-flow or no-reflow, embolisation, or (iv) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

The threshold of cTn values > 99th percentile URL was arbitrarily chosen based on clinical judgement and societal implications of the label of peri-procedural MI. When a cTn value is < 5 x

99th percentile URL after PCI and the cTn value was normal before the PCI—or when the cTn value is  $> 5 \times 99^{\text{th}}$  percentile URL in the absence of ischaemic, angiographic or imaging findings—the term ‘myocardial injury’ should be used. <sup>(3)</sup>

There are a number of cardiac Troponin I assays on the market with wide variability between the manufacturers in commercial characteristics even in fairly new sensitive Troponin assays i.e. Roche versus Abbott, etc. Although the variable assays correlate to some extent, the numeric values can be quite different. The current available assays are not standardised yet and substantial differences exist across methods. This variability prevents comparison between different Troponin results, not only between Troponin T and I, but between Troponin I levels in the published clinical trials. (Table 1.2) In the near future, the above shortcomings could be avoided in the standardised trials with only one manufacturer for Troponin T assays.

Although Troponin is the gold-standard biomarker of heart muscle necrosis due to high sensitivity and specificity and its elevation following PCI does have significant prognostic implications, Creatine phosphokinase MB isoenzyme (CK-MB) elevation is also widely accepted as a biomarker with prognostic significance when raised post-PCI. Ten years ago CK-MB was regarded as the best biomarker for detection of myocardial injury. Its replacement by Troponin was due to high sensitivity of Troponin and allowance for immediate diagnosis of MI in ACS setting even with minor injuries. Elevation of CK-MB above the normal levels occurs in about 30% of patients undergoing elective PCI. <sup>(21)</sup> Among many authorities, measuring CK-MB is still considered a more clinically relevant biomarker for diagnosing type 4a MI. It is agreed that CK-MB, elevations  $> 3-8 \times \text{URL}$ , does have prognostic implications, specifically if accompanied by appearance of Q wave on ECG. <sup>(21,50)</sup>

Therefore when designing the ERIC- PCI trial and writing the study protocol, assessment of CK-MB levels post PCI was included in the protocol.

## Coronary No-Reflow Phenomenon

The no-reflow phenomenon was initially described by Krug et al in 1966<sup>(51)</sup> and detailed further by Kloner in 1974 as an inadequate myocardial perfusion of a given coronary territory despite opening of the artery, without angiographic evidence of mechanical obstruction.<sup>(52)</sup>

In 1986, Bates et al. described the angiographic correlation of no reflow after observing slow contrast flow in the infarct related artery.<sup>(53)</sup> The no-flow/slow-reflow phenomenon manifests as an acute reduction in coronary blood flow in the absence of epicardial vessel obstruction, flow limiting dissection, conduit vessel spasm, or apparent in-situ thrombosis. Interventional (or angiographic) no-reflow, which is actually a myocardial tissue hypoperfusion, can be visualised angiographically in the catheterisation lab in 3.0-4.8% of all PCIs but is most common after acute MI and can reach up to 11.5 %.<sup>(54)</sup> No-reflow is more common in PCI to the vein graft (up to 15%),<sup>(55, 56)</sup> and rotational atherectomy (up to 16%).<sup>(57, 58)</sup> It manifests with low grade thrombolysis in myocardial infarction (TIMI) flow and is typically associated with chest pain and ECG changes.<sup>(57, 59-62)</sup> In these settings, the incidence of no-reflow is between 10% and 20%.<sup>(55,56)</sup> No reflow occurs less commonly after coronary intervention for an acute coronary syndrome (ACS) without ST segment elevation or stable angina.<sup>(55)</sup> Distal embolisation is the main contributing factor to coronary no-flow phenomenon.<sup>(63)</sup> During PCI to vein graft, distal embolisation is universal and occurs regardless of lesion type.<sup>(64)</sup>

In rotablation and PCI to vein graft, plaque gruel can be embolised distally even without ACS and an associated thrombus. The introduction of distal protection devices that filter atherothrombotic debris has provided corroborative evidence that distal microembolisation of plaque and other debris plays a role in microcirculatory ischaemia, particularly in the setting of interventions to vein grafts.<sup>(64)</sup> Trials of rotational atherectomy versus angioplasty have



demonstrated increased incidence of PCI-related MI which translates into increased long-term mortality <sup>(19)</sup> and this is likely explained by embolisation. <sup>(32)</sup> Whether in the setting of an acute vessel thrombosis or not, distal microembolisation appears to contribute to microvascular ischaemia and injury that result in no-reflow. The pathologic sequel of no-reflow is inadequate healing within the ischaemic area, which may inhibit the future development of collaterals. More importantly, following restoration of epicardial blood flow, myocardial reperfusion per se, may cause injury beyond the previous myocardial ischaemic insult. <sup>(63, 65-71)</sup>

## Cardiac MRI Detects PCI-related Myocardial Injury/Infarction

Markedly elevated levels of troponin after PCI are markers of myocardial injury, which can be significant in regards to prognosis.<sup>(25,75)</sup> Debates however still exist about the implications of a small troponin rise after PCI.<sup>(3)</sup> Despite the high sensitivity of cardiac isoenzymes, the diagnosis of myocardial infarction or myocardial injury can still be difficult in patients who had PCI. The extent and degree of myocardial injury after an ischaemic event are strong predictors of patient outcome. Therefore prompt and accurate diagnosis of myocardial injury will have great impact on patients' prognosis.

PCI related myocardial injuries can be assessed by other various methods. From angiographic scores such as TIMI flow grade and imaging techniques such as myocardial contrast echocardiography (MCE), single-photon emission computed tomography (SPECT) or cardiac MRI (CMR).

Myocardial perfusion imaging most commonly performed by <sup>99m</sup>Tc-sestamibi SPECT, is a well established technique in assessment of myocardial ischaemia and significant coronary artery disease. It however has logistical and technical limitations. CMR is the gold standard test to measure infarct size as it provides superior resolution and detects sub-endocardial infarction as well as microvascular obstruction (MVO). First-pass perfusion CMR provides quantitative evaluation of myocardial blood flow.<sup>(72)</sup>

Post-PCI, TIMI myocardial perfusion grade correlates with CMR measures of MVO and infarct size.<sup>(73)</sup> In a recent meta-analysis which assessed 2745 patients of 21 studies, the incidence of MVO detected by CMR was 66%.<sup>(74)</sup>

Due to superiority of CMR in detecting sub-endocardial infarction and less logistical limitations, CMR was chosen as the imaging modality of choice for investigation of peri-PCI MI in the ERIC-PCI trial.

CMR can provide invaluable information in uncertain situations where inappropriate diagnosis of MI post PCI can have multiple clinical, social and financial implications. The underlying rationale is that regional myocardial hypoperfusion and ischaemia lead to a cascade of events, including myocardial dysfunction, cell death and healing by fibrosis.

Although troponin elevation after PCI is common, uncertainties remain about the mechanisms of its release and its relationship to the volume of myocardial tissue loss.<sup>(37)</sup> CMR can identify the significance of troponin rise post PCI. CMR allows non-invasive serial assessment of myocardial function and viability with high resolution. Following intravenous injection of gadolinium contrast agents, gadolinium rapidly distributes into the extracellular space. Contrast-enhanced gadolinium images allow assessment of the transmural extent of irreversible injury and identify sub-endocardial myocardial infarction.<sup>(72, 80, 81)</sup> Furthermore, it permits quantification of even small areas of myocardial necrosis, both due to native coronary disease or after PCI and surgical revascularisation.<sup>(37, 78, 79)</sup> Both acute and chronic infarctions hyperenhance late gadolinium pictures.<sup>(80)</sup> Hyperenhancement occurs in both reperfused<sup>(81-84)</sup> and non-reperfused acute infarcts.<sup>(84)</sup>

Early studies investigating the role of CMR in identifying myocardial injury during PCI (not primary PCI) were done by Selvanayagam et al. in 2005<sup>(37)</sup> and Ricciardi et al<sup>(85)</sup> in 2001 which demonstrated that delayed-enhanced cardiac MRI is able to detect even small myocardial infarcts (micro infarction) in patients who have had elective PCI and CMR, findings which correlated with troponin T and CK-MB elevation. (*Figure 1.1*)

CMR has emerged as the imaging choice to assess the cardioprotective efficacy of novel therapeutic interventions in primary PCI (PPCI)-treated ST elevation MI (STEMI) patients.<sup>(86)</sup> It measures accurate infarct size<sup>(87, 88)</sup> as well as measuring the myocardial salvage index (MSI).<sup>(86, 89)</sup> MSI is a sensitive measure of cardioprotective efficacy, representing the proportion of the myocardium at risk of infarction rescued by a therapeutic intervention - this requires that the myocardium or 'area-at-risk' (AAR) be quantified.<sup>(86)</sup> AAR measurement is an important factor in CMR aided intervention studies. Measurement of the area at risk and infarct size determines myocardial salvage as an indicator of therapeutic benefit in perfusion-injury. The current gold-standard CMR sequence for assessing the myocardial oedema of area at risk (AAR) following an acute coronary event is to use T2-weighted CMR imaging 2-7 days following PPCI to delineate the extent of myocardial oedema.<sup>(86, 90-92)</sup>

Magnetic resonance imaging sequences characterise tissues based on specific nuclear magnetic properties including T1 and T2. T2-weighted images generally show fluids as having high or bright signal intensity, whereas solid tissue like myocardium has intermediate signal intensity. An increase in free water content of tissue increases the signal intensity on T2-weighted images.<sup>(93)</sup> Therefore in acute myocardial injury where myocardial oedema occurs, the area at risk appears slightly brighter.<sup>(94)</sup>

As proven in a few studies, interventions that reduce myocardial injury, significantly improve prognosis.<sup>(95)</sup> RIPC is a novel method, which could reduce injury, occurred during coronary interventions. RIPC could potentially decrease the infarct size, microvascular obstruction and oedema induced during PCI. Therefore CMR scan can provide a comprehensive assessment of the infarct size with robust late gadolinium technique.<sup>(80)</sup> Specifically, in STEMI treated with PPCI, CMR has emerged as the imaging modality of choice to assess the cardioprotective efficacy of novel RIPC.<sup>(96)</sup> In a recent large trial by White et al. in STEMI patients treated by PPCI, RIPC

that was initiated prior to PPCI, reduced myocardial infarct size, increased myocardial salvage, and reduced myocardial oedema. <sup>(86)</sup>

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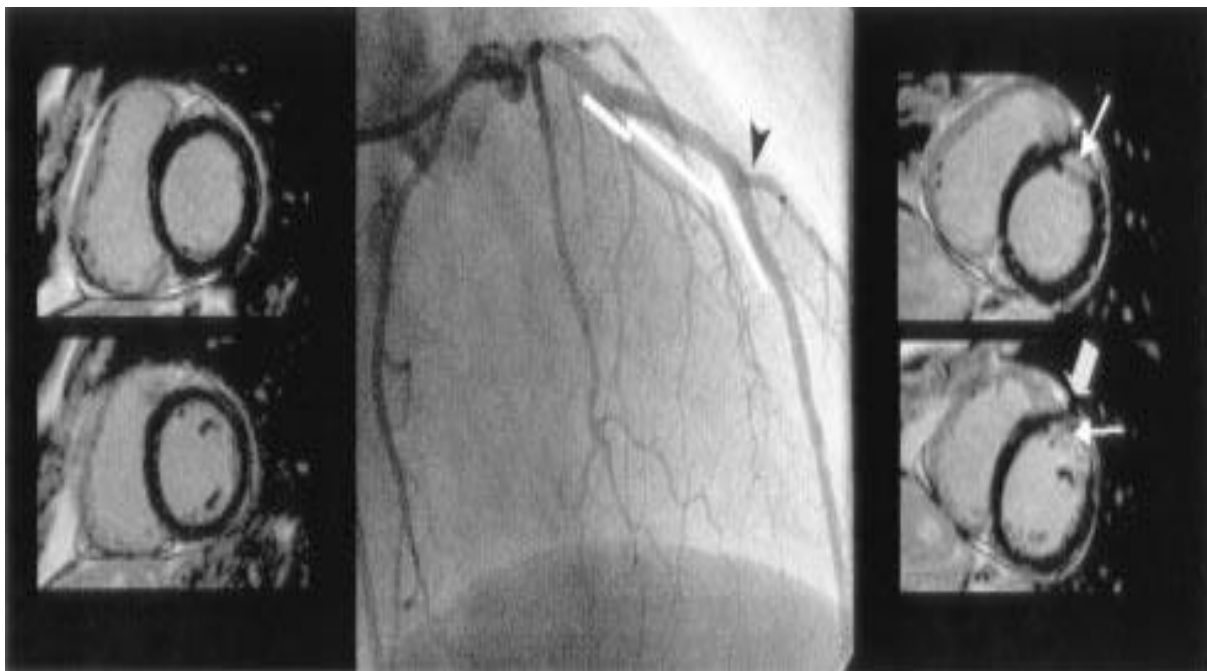


Figure 1.1- Two basal short-axis images (left) in a patient before left anterior descending coronary artery (LAD) PCI showing no LGE. Contrast-enhanced images in the same image plane after PCI (right) reveal new anterolateral wall hyperenhancement (long arrows) adjacent to LAD stent (block arrow). Middle panel shows post-PCI angiogram with position of 3 stents highlighted and good flow in LAD and second diagonal branch (likely affected territory; black arrowhead). Courtesy: Selvanayagam et al. *Circulation* 2005. <sup>(37)</sup>

## Complex PCI

Myocardial injury related to PCI is reportedly higher in procedures, which are so called 'complex'. Complex angioplasty is generally applied to procedures with challenging anatomy when SYNTAX score is  $\geq 23$ , the coronary artery is severely calcified or chronically occluded (CTO) requiring rotational atherectomy, or when the saphenous vein graft (SVG) is severely stenosed and might require laser assisted PCI. The possibility of PCI-related myocardial injury is higher in complex lesions compared with simple single vessel type A lesions. Reducing myocardial injury during PCI is significantly beneficial to patients. Interventions that reduce this injury have been the subject of various researches in recent years.

## What is the SYNTAX Score?

The Syntax score is an angiographic tool grading the complexity of coronary artery disease. The SYNTAX (SYNERgy between PCI with TAXUS™ and Cardiac Surgery) study was organised as an all-comer study for patients with significant lesions in the left main stem and/or the three epicardial coronary arteries.<sup>(97)</sup> The SYNTAX score was specifically developed for this study to prospectively characterise the coronary vasculature with respect to the number of lesions and their functional impact, location, and complexity. Higher SYNTAX scores, indicative of more complex disease were hypothesised to represent a greater therapeutic challenge with potentially worse prognosis. All previous classifications are considered in this classification. A computer program consisting of sequential and interactive self-guided questions calculates the SYNTAX score. The algorithm consists of twelve main questions.

An important characteristic of the SYNTAX score is that it is lesion based. For each lesion a separate score is calculated (Table 1.1). The total SYNTAX score is derived from the summation of these individual scorings. After the completion of the algorithm, a report is automatically generated by the software, summarising all the adverse characteristics and the individual scoring of each lesion as well as the total SYNTAX score. The most important characteristic of the SYNTAX score to be emphasised is that it is focusing on anatomy of coronary vasculature and not on the treatment plan.

**Table 1.1- Twelve essential questions for calculating the syntax score. SYNTAX website**

**1- Dominance**

**2- Number of lesions**

**3- Segments involved per lesion**

*Lesion characteristics*

**4- Total occlusion**

i-Number of segments involved

ii- Age of the total occlusion (>3 months)

iii-Blunt stump

iv-Bridging collaterals

v- First segment beyond the occlusion visible by antegrade or retrograde filling

vi-Side branch involvement

**5- Trifurcation**

i-Number of segments diseased

**6- Bifurcation**

i-Type

ii-Angulation between the distal main vessel and the side branch <70

**7-Aorto-ostial lesion**

**8-Severe tortuosity**

**9-Length>20 mm**

**10-Heavy calcification**

**11-Thrombus**

**12-Diffuse disease/Small vessel**

i-Number of segments with diffuse disease/small vessel



## PCI to Saphenous Vein Graft

Saphenous vein graft (SVG) interventions for older degenerated grafts remain technically challenging, with a high risk for procedural-related events despite advances in therapy; including introduction of mechanical embolic protection devices and administration of potent antiplatelets.

SVGs are often diffusely diseased and contain a significant amount of a friable atherosclerotic material. These atherosclerotic materials increase the risk of distal embolisation, which is the cause of no-reflow phenomenon. No-reflow occurs in up to 15% of cases of PCI to SVG.<sup>(55)</sup> While distal embolisation is virtually universal in SVG intervention, only 15-20% of patients develop MACE (Major Adverse Cardiac Events) in the absence of embolic protection.<sup>(62, 98)</sup> This means embolisation is clinically silent with small emboli. Van Gaal and his colleagues have proven that during angioplasty of vein graft, distal embolisation occurs regardless of the complexity of lesion and procedure.<sup>(99)</sup> Therefore distal protection devices are recommended for PCI to vein graft procedures. However, it has been noted that not all SVG lesions are amenable to distal protection device (DPD), and in 33%–57% of vein graft PCIs, lesion location or the lack of a landing zone may not permit use of protection devices.<sup>(99)</sup>

Laser ablation catheter is another promising application for handling highly thrombotic materials in these lesions. This technique is associated with rapid removal of thrombus, debulking of the underlying plaque, and reduction in the risk of distal embolisation.<sup>(100)</sup> Emboli protection devices, laser application and administration of GP IIb/III are all proven to be beneficial but adverse events still occur. Novel approaches such as RIPC may play a big protective role here.

## Rotational Atherectomy

Treatment of heavily calcified coronary arteries is still a challenge in interventional cardiology. Balloon passage, balloon inflation and stent deployment in heavily calcified lesions, which are often fibrotic and undilatable, could be impossible. Attempts to tackle these resistant lesions with high-pressure balloon inflations greatly increase the risk of dissection and complications. Incomplete stent expansion and stent apposition in the setting of extensive calcification increases the risk of sub-acute stent thrombosis<sup>(101)</sup> and in-stent restenosis,<sup>(102)</sup> therefore, it is essential to use the best technique for a desirable result. A number of devices and techniques have been designed to overcome the difficulties posed by calcium.

High frequency rotational atherectomy is one of the niche devices developed based on the ablation of atherosclerotic plaques. Rotational atherectomy that was introduced in late 1980s is a common invasive method for modifying the calcified lesions prior to the angioplasty and stent implantation. First few experiments were performed on animal models. Fourrier and his colleagues performed the first case of rotablation in human coronary arteries in 1988.<sup>(103)</sup> Physical principal of RA is the selective and differential cutting of the inelastic material while maintaining the integrity of the elastic tissue. Rotablation crushes the calcified plaque by high-speed (140 000–180 000 revolution per minute) diamond-coated burr but preserves the normal elastic tissues of the walls; hence it is safe on normal tissue. Burr size should not exceed a burr/artery diameter ratio of 0.8. Aggressive rotational atherectomy (defined as burr size >2.25 mm or burr/artery diameter ratio >0.8) has been shown to increase procedural-related myocardial injuries and should be avoided. This is based on the results of the following trials: COBRA study<sup>(104)</sup> to Determine Rotablator and Transluminal Angioplasty Strategy (STRATAS)<sup>(105)</sup> and Coronary Angioplasty and Rotablator Atherectomy Trial (CARAT).<sup>(61)</sup>

In theory, the mechanical debulking of atherosclerotic plaques with the use of rotational or direct coronary atherectomy devices prior to stent placement would be beneficial in three ways: (1) the risk of in-stent restenosis would be lowered by decreasing the underlying plaque burden<sup>(106)</sup>, (2) the risk of in-stent restenosis would also be lowered because of an increase in the acute procedural minimal luminal diameter (MLD), and (3) there would be a decrease in the risk of abrupt closure because of the preservation of the original arterial size and decreased barotrauma to the vessel.

The majority of the microparticle debris generated from rotational atherectomy are between 5-10 µm in diameter, which often do not significantly impact coronary blood flow and are eventually cleared by the reticuloendothelium system.<sup>(107)</sup> However, these particles may have a detrimental effect on the myocardial microcirculation.

Rotablation is not free from complications. Side branch occlusion, downstream embolisation, dissection, MVO and spasm could affect the prognosis significantly. Slow flow/no-flow is one of the most serious complications of rotational atherectomy.

Coronary slow flow/no-reflow is defined as a decrease or cessation of blood flow in the absence of an apparent occlusive dissection or spasm and is believed to occur as a result of distal microparticle embolisation. Rotational atherectomy increases the risk of no-flow especially in heavily calcified lesions.<sup>(57, 58, 108)</sup> Procedural technique and operator's skill is very important and plays an important role in preventing this complication.<sup>(108)</sup>

Platelet activation caused by rotational atherectomy, is dependent on rota burr speed.<sup>(109)</sup> Hence, it is advised not to increase the speed to more than 150,000 rpm. Strict avoidance of significant drops in rpm is recommended. Also it is advised to start with smaller burr size and to engage with the lesion for short a period of time only.<sup>(65)</sup> The vessel should be continuously flushed with normal saline containing GTN, verapamil and heparin.<sup>(110)</sup>

## Chronic Total Occlusion

Chronic Total Occlusion (CTO) is defined as a 100% coronary artery occlusion; with TIMI flow grade equal to 0 and duration of more than 3 months. CTO recanalisation represents one of the most challenging and technically demanding aspects of percutaneous treatment of coronary artery disease.

Chronic total occlusion of coronary arteries is frequently (18–30%) encountered on diagnostic coronary angiograms. A recent study showed that advancing age increases the likelihood to detect a CTO on diagnostic coronary angiography. <sup>(111, 112)</sup>

Technical advances in the design of angioplasty equipment particularly of specialised wires and also enhanced operators' skills have increased recanalisation rates of CTOs, but the success rate is still lower in comparison with conventional PCI of non-occluded arteries. <sup>(113, 114)</sup> This, in most cases is due to an inability to pass the guide-wire through the area of tight stenosis, or due to risk of coronary perforations and in-hospital adverse events. Traditional fears of such procedure complications, makes operators less willing to approach these lesions and opt for medical management or surgery. But in fact in experienced hands, CTO recanalisation is feasible and relatively safe, even in patients with high-complexity lesions and clinical characteristics denoting higher risk, for whom more benefits would be expected from such a procedure. Long-term outcomes of CTO PCI have been improved because the widespread introduction of stent utilisation, which is associated with reduced rates of restenosis and re-occlusion when compared with balloon-only angioplasty.

PCI-related myocardial injury/infarction is among the most common complications of CTO PCI. However, in most cases cardiac biomarker elevation are mostly asymptomatic. The MI rates in a study performed recently by Patel et al. <sup>(115)</sup> ranged widely from 0% to 19.4%, likely reflecting

significant variability in the frequency of systematic cardiac biomarker measurement after PCI. This finding was consistent with previously published data from the National Cardiovascular Data Registry, in which a median of only 7% of patients had cardiac biomarker measurements after PCI.<sup>(116)</sup>

Despite the high plaque load of organised thrombotic material in CTOs, the incidence of cardiac biomarker elevation after recanalisation of CTOs is similar to that after stenting of single non-occlusive lesions.<sup>(117)</sup>

Coronary perforation is among the most feared complications of CTO PCI, due to the risk of tamponade. CTO PCI carries increased risk of perforation due to routine use of stiff and polymer-coated guidewires and frequent uncertainty about the vessel course. The incidence of coronary perforation in non-CTO PCI is approximately 0.19% and occurs more commonly in heavily calcified tortuous vessels using hydrophilic wires and atheroablative devices.<sup>(115)</sup>

## **Prophylactic Measures to Reduce PCI-related Myocardial Injury**

No-reflow is usually treated with vasodilators, such as calcium channel blockers (verapamil, diltiazem, or nicardipine), adenosine or nitroprusside, which have their effect on the microcirculation. Many catheterisation laboratories routinely use a cocktail of nitroglycerin, verapamil, and heparin in the flush solution that has been shown to reduce the incidence of spasm and slow flow.<sup>(110, 117)</sup>

These pharmacological agents as well as glycoprotein IIb/IIIa (GP IIb/IIIa) inhibitors modify no-reflow and slow-flow complications but adverse outcomes still persist. GP IIb/IIIa inhibitors have been associated with a 50% reduction in cardiac enzymes elevation during the procedure, as well as a reduction in burr induced platelet aggregation during rotational atherectomy.<sup>(118, 119)</sup>

This beneficial effect of the GP IIb/IIIa inhibitors signifies the importance of the activation of platelets and their interaction with atheromatous debris in causing slow flow and other adverse procedural events during rotablation.<sup>(120)</sup> Unfortunately administration of GPIIb/IIIa is not free from adverse side effects. Specifically in angulated heavily calcified lesions where the risk of perforation and bleeding could increase.<sup>(120)</sup>

### **A) Embolic Protection Devices**

Increased rates of PCI-related complications resulting from distal embolisation of atherosclerotic debris from SVGs<sup>(55)</sup> resulted in the advent of distal microcirculatory protective systems. Distal protection devices are divided into two main categories: (i) those that occlude the distal artery with a balloon on a catheter and aspirate atherosclerotic debris and thrombus with a small

catheter just proximal to the balloon, and (ii) those that have a distal umbrella-like device to trap embolised debris.

More recently a proximal balloon occlusion device has been introduced using a similar concept to the distal balloon catheter, with aspiration of debris through the device before the protective balloon deflation.<sup>(64)</sup> SVGs histologically have comparatively more foam and inflammatory cells and less calcification than native coronary lesions, with resulting plaques being more friable and likely to embolise. Coronary intervention techniques utilising the distal mechanical filter system have recently been shown to have beneficial results when intervening on SVGs as compared to using standard angioplasty techniques. In the first large randomised trial of a distal embolic protection device used in this group of lesions, there were less major adverse cardiac events at 30 days and less no-reflow phenomenon.<sup>(98)</sup> A recent review of the devices and the use of these in numerous subsequent clinical trials in SVG interventions concluded that use of embolic protection devices resulted in 40–50% reduction in embolisation, and significant decrease in procedural-related major adverse cardiac events. Distal protection however, does not entirely eliminate no-reflow.<sup>(121)</sup>

## **B) Pharmacotherapy**

### **Antiplatelet Therapy and Anti-Coagulation**

Inhibition of platelet activation is fundamental in the management of PCI-related myocardial injury. Recognising that coactivation of platelets and the blood coagulation cascade contributes to the pathophysiology of arterial thrombosis, monotherapy with either an antiplatelet agent or

an anticoagulant may be insufficient for maximising the prevention and treatment of platelet-rich arterial thrombosis which can induce myocardial injury.<sup>(122)</sup>

Combination of aspirin and clopidogrel (a thienopyridine) is the gold standard treatment in Acute Coronary Syndrome (ACS) and PCI with stent insertion.<sup>(123-127)</sup>

Current guidelines recommend 4 weeks treatment with clopidogrel in case of BMS insertion and 12 months treatment with clopidogrel when DES is inserted. Most stent thromboses occur early (<30 days) or very late (>1 year).<sup>(128-130)</sup> Compared with BMS, fewer thromboses are observed during the first year of DES implantation, but more thromboses beyond one year after PCI.<sup>(130, 131)</sup> Stopping treatment with a thienopyridine ADP-receptor antagonist causes a >10-fold increase risk of stent thrombosis.<sup>(132)</sup>

Anticoagulation with heparin is essential during angioplasty. It avoids thromboembolic complications as well thrombotic complications of the intervention.<sup>(132)</sup>

Aggressive antiplatelet therapy with aspirin and clopidogrel reduces both short- and long-term adverse cardiovascular events per se or PCI related ones.<sup>(123, 133, 134)</sup> There remains, however, an unacceptable high rate of residual recurrent ischaemic events among such patients.<sup>(123, 124)</sup>

Clopidogrel, a thienopyridine derivate of the second generation, is an adenosine diphosphate (ADP) receptor antagonist. It selectively inhibits the binding of ADP to its platelet receptor (P2Y<sub>12</sub>) and the subsequent G protein linked mobilisation of intracellular calcium and activation of the GP IIb/IIIa complex.<sup>(135)</sup>

Variations in the therapeutic response to standard doses of clopidogrel are now well recognised.<sup>(136,137)</sup> Platelet inhibition varies between patients in a normal bell-shaped distribution<sup>(138-140)</sup> with up to 30% of patients treated with clopidogrel not having an adequate antiplatelet response.<sup>(141-143)</sup> This variability was also found to be true with Ticlopidine.<sup>(143)</sup>



Studies suggest that Clopidogrel resistance might be due to individual variability in platelet responses to adenosine diphosphate.<sup>(144)</sup>

The novel platelet P2Y<sub>12</sub> receptor antagonists, prasugrel and ticagrelor both have demonstrated superior and more consistent levels of P2Y<sub>12</sub>-mediated platelet inhibition when compared with clopidogrel, with a commensurate 20% relative reduction in these ischaemic events but at the cost of increased bleeding events.<sup>(145-147)</sup>

## **Glycoprotein IIb/IIIa Inhibitors**

As per recommendation by the NICE guideline, GP IIb/IIIa inhibitor can be considered as an adjunct to PCI for all patients with diabetes undergoing elective PCI, and for those patients undergoing complex procedures.

Abciximab is a monoclonal antibody that targets the GP IIb/IIIa receptor on the surface of platelets. Tirofiban is a non-peptidal antagonist of the GP IIb/IIIa receptor and is one of the small-molecule GP IIb/IIIa inhibitors. It prevents fibrinogen from binding to the GP IIb/IIIa receptor, thus blocking platelet aggregation.

## **Adenosine**

Various pharmacological vasodilators have been associated with an improvement in PCI outcome.<sup>(60, 147, 148)</sup> Adenosine appears to play an essential counter-regulatory compound role in the maintenance of microcirculatory flow, due to its numerous pharmacological actions. Adenosine could decrease mechanical obstruction of capillary channels caused by neutrophil

adherence and neutrophil-mediated cellular damage. The potent arteriolar vasodilator properties of adenosine would oppose the effects of vasoconstrictor substances present in vascular bed, such as endothelin, leukotrienes.<sup>(149)</sup>

The ability of adenosine to reduce inflammation by inhibiting multiple cell types involved in cellular immunity may also contribute to tissue protection.<sup>(149)</sup>

Sdringola et al.<sup>(150)</sup> examined the efficacy of adenosine boluses (24 µg each) to reverse slow/no-reflow. Reversal of slow/no-reflow was observed in 91% of patients who received high doses of adenosine ( $\geq 5$  boluses) and only in 33% of those who received low doses ( $< 5$  boluses). Moreover, the final TIMI flow was significantly better in the high dose group. Other authors suggested that combined therapy with adenosine and nitroprusside<sup>(30)</sup> or nicorandil<sup>(31)</sup> might provide better improvement in coronary flow compared to intracoronary adenosine alone in case of impaired flow during coronary interventions.

## **Intra-Coronary Verapamil**

The role of this vasodilating agent in prevention of MVO and improvement of the coronary flow has been studied in a few randomised controlled trials. It is confirmed that intracoronary and intragraft administration of verapamil can improve the flow in more than 80% of patients whose PCI procedure was complicated with no-reflow, including degenerative vein graft intervention.

<sup>(54)</sup> <sup>(151, 152)</sup> Apart from vasodilation, verapamil may inhibit platelet aggregation and thrombus formation in the microvasculature.<sup>(153)</sup>

## Intra-Coronary Nitroprusside

The third most commonly administered vasodilating drug is intracoronary nitroprusside. Nitroprusside alone improves flow in 75% of no-reflow patients.<sup>(154)</sup> In general, intracoronary verapamil, diltiazem, or nitroprusside can be used initially for the treatment of no-reflow. However, in comparison to adenosine, verapamil and nitroprusside have failed to reduce adverse outcomes occurring in the setting of no-reflow.<sup>(155)</sup> There is no consensus when it comes to exact dosing of vasodilators for the treatment of no-flow.

## Nicorandil

Nicorandil dilates coronary microcirculation, induces ischaemic preconditioning,<sup>(156)</sup> is antiarrhythmic, improves LV function and reduces reperfusion injury via the adenosine triphosphate (ATP)-sensitive K<sup>+</sup> channel.<sup>(157, 158)</sup> Nicorandil is a K<sup>+</sup><sub>ATP</sub> channel opener: Nicorandil activates K<sup>+</sup><sub>ATP</sub> channel, causing K<sup>+</sup> efflux. This action, hyperpolarises the cell that inactivates voltage-gated calcium channels and reduces free intracellular Ca<sup>2+</sup>. Studies in animals have demonstrated that Nicorandil encourages myocardial recovery, reduces infarct size, and possibly decreases neutrophil activation to exert a cardioprotective effect.<sup>(159)</sup> Both oral and intravenous administration of nicorandil may reduce the incidence of microvascular dysfunction after elective and PPCI by dilating coronary resistance vessels.<sup>(160-163)</sup> In a study published in 2013, in patients undergoing PCI for stable angina, Troponin elevation more than fivefold the normal range was significantly larger in the control group than in the Nicorandil group.<sup>(164)</sup> Single oral dose of Nicorandil prior to elective PCI could also reduce PCI-related myocardial injury.<sup>(165)</sup>

## **Intra-Coronary Nitrate**

Intra-coronary nitrate is indicated during percutaneous transluminal coronary angioplasty (PTCA) to facilitate prolongation of balloon inflation and to prevent or relieve coronary spasm. Intra coronary nitrate is frequently used during coronary interventions to improve the coronary flow in low flow or no-reflow condition.<sup>(162, 166-168)</sup> The primary effect of nitroglycerin on the coronary artery system is dilating the epicardial coronary arteries.

Nitrate influences the oxygen supply to the ischaemic myocardium by causing redistribution of blood flow along collateral channels and from epicardial to endocardial regions by selective dilatation of large epicardial vessels. It reduces the requirement of the myocardium for oxygen by increasing venous capacitance, causing a pooling of blood in peripheral veins, thereby reducing ventricular volume and heart wall distension.<sup>(169)</sup>

## Interventions and Strategies to Protect Myocardium

The concept of increasing the tolerance of myocardium against the deleterious effects of myocardial infarction was initiated and investigated in a laboratory setting in 1970 by Maroko et al. <sup>(170)</sup> It was proposed that beta blockers, glucose-insulin-potassium, or hyaluronidase at the time of coronary occlusion could reduce the size of the resulting infarction in the canine experiment. None of these proposed interventions seemed to be effective though. Yellon and his colleagues believed that perhaps the models for evaluating infarct size were sub-optimal at the time. <sup>(171)</sup>

The idea however, initiated further search for probable interventions that could protect the myocardium in clinical setting. A few years later, Jennings and Reimer demonstrated that reperfusion was the crucial therapeutic strategy to protect the ischaemic myocardium, and soon after, thrombolytic therapies became a routine to reduce the infarction size. <sup>(172)</sup> During ischaemia, anaerobic metabolism develops and results in decrease of ATP production. There is insufficient available energy to maintain cell membrane pump activity, antioxidant defences, pH and calcium homeostasis, and mitochondrial integrity. These and other consequences of ischaemia inevitably lead to cell death, unless blood flow is restored.

Reperfusion can paradoxically be harmful though, as it can exacerbate the necrotic component of cell death as evidenced by an extension in infarct size, following a fixed period of ischaemia. <sup>(173, 174)</sup> Reperfusion with oxygenated blood is essential for any tissue salvage, but the sudden rush of oxygen produces reactive oxygen species and oxidative stress. This reperfusion method was indeed reducing the infarct size, but was not ideal i.e. myocardial salvage was not fully achieved; hence a novel approach (conditioning) to rescue the myocardium was sought after.

## Conditioning the Heart

It was in 1986 when Murry et al. discovered that the heart has got an intrinsic and powerful mechanism to protect itself from harmful injuries. This mechanism was named “preconditioning”.<sup>(175)</sup> This phenomenon whereby transient and brief periods of ischaemia confer protection against a subsequent prolonged and injurious period of ischaemia, is now recognised as “the strongest form of in vivo protection against myocardial ischaemic injury other than early reperfusion”.<sup>(176)</sup>

Inducing brief non-lethal episodes of ischaemia and reperfusion to the heart, either prior to, during, or immediately after an episode of sustained lethal myocardial ischaemia reduces myocardial injury by involving wide range of cells and pathways. It is possible that such a protective mechanism occurs naturally in humans. Pre-infarct angina may resemble preconditioning effect. In analysis of the TIMI 4 study, patients who had previous angina or angina that occurred immediately prior to myocardial infarction had lower rate of in-hospital death, severe congestive heart failure (CHF) or shock.<sup>(177)</sup>

Several methods for conditioning of the myocardium have been introduced. These innate cardioprotective mechanisms are not just limited to direct ischaemia of the heart i.e. it is not necessary to condition the heart directly.

If the conditioning stimulus is performed prior to ischaemic insult, it is called pre-conditioning. Ischaemic per- or post-conditioning means the ischaemia is applied during or following the ischaemia. Local preconditioning occurs when the conditioning stimulus is applied to the same organ or tissue that will subsequently face the ischaemic insult i.e. myocardium prior to ischaemia. This method is called Remote Ischaemic Preconditioning (RIPC), when stimulus is

applied to a distant organ or tissue, which then protects against index ischaemia. It can also act through pharmacological manipulation.

However, ischaemic preconditioning has not been translated into the clinical setting, not only because it is impossible to predict clinical acute coronary syndromes, but also, even in the setting of predictable ischaemic/reperfusion injury such as PCI, there are practical difficulties in delivering the preconditioning stimulus to the myocardium.<sup>(178)</sup> How the heart remembers that it has been preconditioned is another mystery that has resisted laboratory investigations.

Clinical application of ischaemic preconditioning was first reported in 1993 by Yellon et al, who studied patients undergoing coronary artery bypass grafting.<sup>(179)</sup> After putting the patients on cardiopulmonary bypass, the aorta was cross-clamped twice for 3 minutes with an interval of 2 minute. Distal bypass anastomosis with a technique of intermittent cross-clamping was then performed under electrically induced ventricular fibrillation. It was shown that the myocardial ATP level after the procedure was twice as high as that of the control group.

In the next few paragraphs, ischaemic preconditioning will be explained in details.

## **Preconditioning with Ischaemia (classic form)**

Murry, Jennings and Reimer in 1986 discovered an intrinsic mechanism of cardioprotection and named it "Ischaemic Preconditioning".<sup>(176)</sup> In their experiment, brief intermittent ischaemia and reperfusion induced protection against a subsequent sustained ischaemic episode. To achieve this conclusion, they performed two sets of experiments. In the first set, one group of dogs (n = 7) was preconditioned with four 5 minutes of circumflex occlusions, each followed by 5 minute of reperfusion, and then 40 minutes of sustained occlusion was performed. The control group (n = 5) received a single 40-minute circumflex artery occlusion. In the second study, an identical preconditioning protocol was followed, and animals (n = 9) then received a sustained 3-hour occlusion following preconditioning. Control animals (n = 7) received a single 3-hour occlusion without prior preconditioning. Animals were allowed 4 days of reperfusion thereafter. Histologic infarct size then was measured and was related to the major baseline predictors of infarct size, including the anatomic area at risk and collateral blood flow.<sup>(176)</sup>

In the 40-minute study, the histological post mortem infarct size was paradoxically limited to 25% of that seen in the control group ( $p < 0.001$ ) following ischaemic preconditioning i.e. those that were preconditioned suffered only one-quarter as much necrosis as that incurred in non-preconditioned hearts. In the 3 hour study, there was no difference in infarct size between the pre-conditioned and control groups.



## Early vs. Late Preconditioning

Remote Ischaemic Preconditioning has a biphasic pattern of myocardial protection. The cardioprotection described by Murry et al. expired after a few hours and is known as “classic” or “early” ischaemic preconditioning.<sup>(180)</sup> This initial protection was transient and acted within a few minutes to 2-3 hours after the preconditioning stimulus. Later on, it was discovered that a delayed form of the protection recovers after 24 hours of the preconditioning stimulus, and has been referred to as the second window of protection (SWOP).<sup>(181-183)</sup> SWOP occurs after 24 hours and lasts up to 72 hours after a preconditioning stimulus.<sup>(184)</sup> Both classical and SWOP preconditioning share some similarities.<sup>(181)</sup> In both cases the preconditioning ischaemia provokes the release of a number of trigger substances that interact with cell surface receptors, thereby initiating a signalling cascade of events. It seems that the protective effect within the second window is at least as powerful as that of classic ischaemic conditioning.<sup>(184)</sup>

MURRY *et al.*

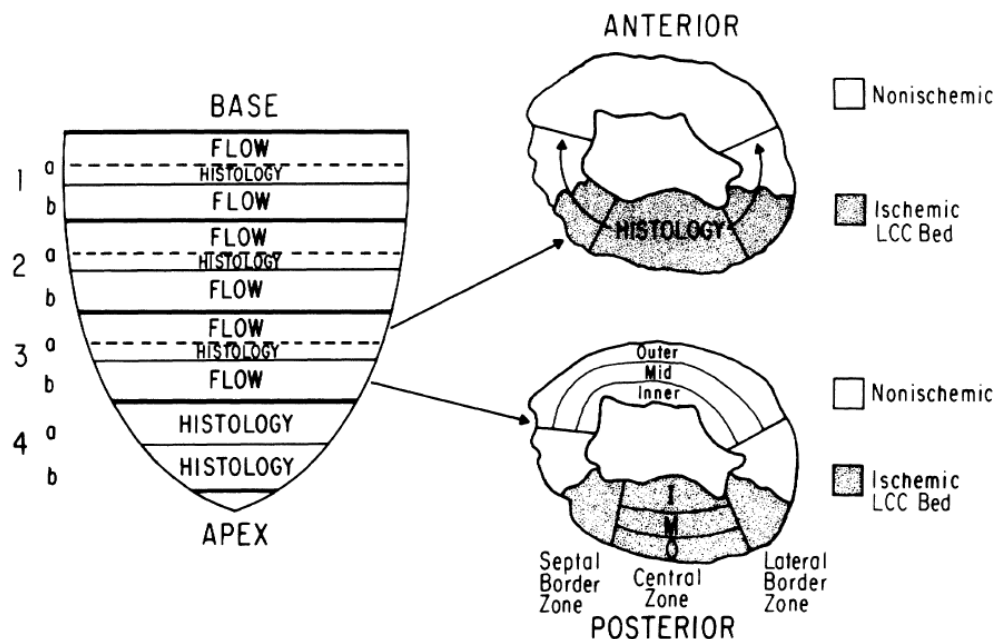


Figure 1.2- Reimer and Jennings. Circulation 71:1069. 1985 The post mortem technique used by Murry et al. for calculating area at risk, infarct size and regional blood flow. The LV was sliced into 4 and then to 8 slices.

## Duration and Intensity of Preconditioning

As seen above, not all combinations and durations of ischaemia and reperfusion will trigger the preconditioning phenomenon and protect ischaemic myocardium. A critical threshold appears to be present. One to two minute duration is not enough and effective for preconditioning. <sup>(185)</sup> Apart from the number of the preconditioning cycles and the duration of the ischaemia, the duration of the intermittent reperfusion determines the protection achieved by ischaemic preconditioning. The most interesting part of this preconditioning process is Memory. The myocardium 'remembers' that it has been preconditioned, even if the ischaemic stimulus has occurred up to several hours before the actual ischaemia. The exact nature and location of this memory is one of the great unsolved mysteries of ischaemic preconditioning. <sup>(186)</sup>

## Remote Ischaemic Preconditioning

Ischaemic preconditioning and ischaemic postconditioning require the intervention to be applied directly to the heart which may not be clinically practical. Hence, remote ischaemic conditioning which provides a more easily applicable method of myocardial protection has attracted the attention of investigators for more than 20 years.

In RIPC, applying one or more brief cycles of alternating ischaemia and reperfusion to an organ or tissue away from the heart protects the myocardium against the detrimental effects of a lethal episode of ischaemic reperfusion injury. The idea of protecting the heart by causing brief ischaemia on a different vascular setting away from the actual territory was first developed by Karen Przyklenk and colleagues in 1993 in an experimental setting.<sup>(187)</sup> They demonstrated that repeated episodes of occlusion–reperfusion (4 times for 5 minutes followed by 5 minutes of reperfusion) of the circumflex coronary artery prior to one-hour occlusion of the left anterior descending coronary artery, reduced the infarct size in the left anterior descending territory of dogs ( $6\pm 2\%$  versus  $16\pm 5\%$  of the area at risk,  $P<0.05$ ). This was actually, remote intracardiac conditioning.<sup>(188)</sup>

This discovery that the conditioning stimulus could be applied to an organ or tissue away from the heart was a major breakthrough in applicability of preconditioning and has facilitated the translation of cardioprotective strategy into the clinical practice. The concept of preconditioning has since been expanded to a more clinically amenable strategy with the introduction of remote ischaemic preconditioning (RIPC), in which the preconditioning episodes are applied to an organ or tissue remote from the heart.

Przyklenk's findings were extended further and was noted that myocardial protection could also be achieved by intermittent interruption of the blood flow in a non- cardiac tissue. Studies

confirmed that brief ischaemia applied to the kidney,<sup>(189)</sup> intestine GHO,<sup>(190)</sup> or skeletal muscle<sup>(191)</sup> could also protect the heart against a subsequent myocardial infarction. Evidence from various results shows that in animals, transient ischaemia of a wide range of tissues induces a systemic effect with multiorgan protection (including the brain) against subsequent extended ischaemia-reperfusion injury.<sup>(192-199)</sup>

Birnbaum and colleagues<sup>(200)</sup> were first to show that remote ischaemic preconditioning by transient lower limb ischaemia prior to acute coronary syndrome (ACS) could reduce myocardial infarct size by 65% in animals. The invasive stimulus consisted of a partial reduction in femoral artery flow applied in conjunction with electrical stimulation of the leg muscle. This phenomenon was named 'IPC at a distance.'

Subsequently, a far less invasive and simpler procedure for inducing transient upper limb ischaemia as a remote ischaemic preconditioning stimulus was done with human participants.<sup>(201)</sup> In 2002, Kharbanda and MacAllister pioneered the use of non-invasive blood pressure (BP) cuff in human volunteers. In their RIPC protocol, the alternating ischaemia and reperfusion comprised the inflation and deflation of a BP cuff placed on the upper arm to apply three 5 min cycles of alternating ischaemia and reperfusion to skeletal muscle of the forearm.<sup>(202)</sup> Four cycles of 5 min of ischaemia followed by 5 min of reperfusion of the arm protected against endothelial dysfunction induced by subsequent long-lasting ischaemia in the other arm. In a second part of the study, a similar preconditioning stimulus to the hind limb protected against myocardial infarction in pigs undergoing 40 minutes occlusion of the left anterior descending coronary artery.<sup>(202)</sup> The cardioprotective end points conferred by RIC include infarct size reduction, improvement of ATP recovery post-ischaemia,<sup>(201)</sup> anti-arrhythmia,<sup>(203)</sup> and improvement of ventricular contractile function.<sup>(201)</sup>

The timing of the RIC stimulus can accommodate most clinical settings of acute ischaemic/reperfusion injury. This stimulus can be applied to either upper limb or lower limb either prior to ischaemia/reperfusion (remote ischaemic pre-conditioning, RIPC) or even at the time of myocardial reperfusion (remote ischaemic post-conditioning). Limb occlusion by tourniquet or blood pressure (BP) cuff is especially relevant for clinical application because it is a safer non-invasive and inexpensive procedure.

Kharbanda and colleagues <sup>(202)</sup> have shown that the RIPC protocol is capable of reducing myocardial injury as measured by troponin-I release in a porcine model of coronary artery bypass graft surgery. RIPC induced by brief periods of limb ischaemia using a blood pressure cuff or tourniquet, reduced myocardial infarction by 50% in a porcine model and protected against ischaemia/reperfusion injury when performed in the recipient animal in experimental cardiac transplantation.<sup>(202, 204)</sup> In a pre-clinical experimental model of cardiac bypass surgery, Kharbanda and his team showed reduced myocardial injury and improved lung function after a clinically relevant period of cross-clamping and cardiopulmonary bypass.<sup>(202)</sup>

## **Beneficial Clinical Effect of RIPC in CABG and PCI Setting**

Demonstrating that transient ischaemia—of intra-abdominal organs—could protect the heart against myocardial infarction was essential for development of the idea of remote preconditioning. Simplicity and cost effectiveness of RIPC, have facilitated a rapid translation to clinical trials.

Revascularisation by the means of Coronary Artery Bypass Graft surgery (CABG) is one of the most established methods in management of severe ischaemic heart disease. Despite advances in surgical technique, which enable more protection of myocardium during surgery, the average mortality remains around 1.6 % in the UK. Despite cardioplegic arrest, the incidence of perioperative myocardial infarction remains high at 9.8%. One of the major causes of myocardial injury during cardiac surgery is acute ischaemia-reperfusion as a consequence of aorta cross clamping. The clinical need to reduce this injury and induce better protection, especially in those with high-risk status (i.e. advanced age, diabetes mellitus, and prolonged cross-clamp time), has encouraged scientists and clinicians to undertake projects which increase myocardial tolerance to sustained ischemia, in particular preconditioning.

The first study of remote preconditioning by transient limb ischaemia showed no effect on CK release in adults undergoing coronary bypass surgery.<sup>(205)</sup> These results however, cannot be considered definitive as the preconditioning stimulus consisted of only two cycles of 3 minute of ischaemia and 2 minute of reperfusion of the arm, and there were only four patients in both the control and preconditioning groups.

The first successful clinical application of RIPC was reported in 2006 in 37 children undergoing corrective cardiac surgery for congenital heart disease.<sup>(206)</sup> The release of Troponin I was lower in the preconditioned group. The postoperative inotrope score and airway resistance in this group

were lower than they were in the control group. This study was followed by a few other trials in cardiac surgery including CABG and valve operation, which are summarised in Table 1.3. As shown, the outcome of these trials is not consistent.

Regarding the PCI setting, it is well recognised that myocardial injury is an adverse event of PCI. Numerous researchers have investigated the efficacy of RIPC in PCI, in acute or elective setting. RIPC could potentially confer different forms of cardioprotection including reducing infarct size, lethal arrhythmias, and cardiac dysfunction following ischaemic injury. Although a few RIPC trials have shown favourable effects on myocardial ischemia/reperfusion injury in PCI, the results have been inconsistent so far. (Tables 1.2)

Probable reasons of these conflicting outcomes are discussed in details in Chapters 5 and 6.

**Table 1.2- RIPC clinical trials to date in PCI setting**

Year	Author	Clinical setting	Intervention	Outcome
2017	Yilmaztepe <sup>(207)</sup>	Elective PCI	Upper arm 1 x 5 min	Lower Trop I in RIPC group
2017	Ladejobi <sup>(208)</sup>	PPCI	Upper arm 4 x 5 min	Reduced serum BNP and HF
2014	Zografos <sup>(209)</sup>	Ad hoc PCI	Upper limb 1 x 5 min	Lower Trop I in RIPC group
2014	Sloth <sup>(210)</sup>	PPCI	Upper limb 4 x 5 min	Lower MACCE in RIPC group
2014	Liu <sup>(211)</sup>	Elective PCI	Upper limb 3 x 5 min	Lower Trop I, CK, CK-MB in RIPC group
2014	Manchurov <sup>(212)</sup>	PPCI	Upper limb 4 X 5 min	Improved endothelial function
2013	Davies <sup>(213)</sup> (CRISP Stent f/u)	Elective PCI	Arm 3 x 5 min	MACCE-free survival both short- and long-term f/u
2013	Ahmed <sup>(214)</sup>	Elective PCI	Arm 3 x 5 min	Lower Trop-T in RIPC group
2013	Prasad <sup>(215)</sup>	Elective PCI	Arm 3 x 3 min	No difference in Trop T release in sham and RIPC groups
2012	Ghaemian <sup>(216)</sup>	Elective PCI	Lower limb 2 x 5 min	Lower Troponin T in RIPC group
2012	Luo <sup>(217)</sup>	Elective PCI	Arm 3 x 5 min	Lower Troponin I in RIPC group
2011	Munk <sup>(218)</sup>	PPCI	Arm 4 x 5 min	Non significant improvement in LV Systolic function in RIPC group
2010	Botker <sup>(96)</sup>	PPCI	Arm 4 x 5 min	Increased myocardial salvage index in RIPC group
2010	Rentoukas <sup>(219)</sup>	PPCI	Arm 3 x 4 min plus morphine	Full ST segment resolution in RIPC plus morphine group
2009	Hoole <sup>(15)</sup>	Elective PCI	Arm 3 x 5 min	Lower Troponin I in RIPC group
2006	Iliodromitis <sup>(220)</sup>	Elective PCI	Both arms 3 x 5 min	Increased Top-I, CK-MB and CRP in RIPC group



**Table 1.3- RIPC trials to date in adult cardiac surgery**

Year	Author	Clinical Setting	Intervention	Outcome
2015	Min <sup>(221)</sup> (RISPO sub-study)	CABG	Upper limb 4 x 5 min	Improved Post-op 24 hr PaO <sub>2</sub> /F <sub>I</sub> O <sub>2</sub>
2015	Hausenloy <sup>(222)</sup> (ERICCA)	On-pump CABG +/- valve	Upper limb 4 x 5 min	Noimprovement in clinical outcome
2015	Meybohm <sup>(223)</sup> (RIPHeart)	CABG	Upper limb 4 x 5 min	No improvement in clinical outcome
2014	Healy <sup>(224)</sup> The RIPC trialists	All Cardiac Surgery	RIPC	No significant effect on endpoint
2014	Kottenberg <sup>(225)</sup>	CABG in Sulphonyl-urea treated diabetics	Upper limb 3 x 5 min	Reduced Troponin in non-diabetic group
2014	Yang <sup>(226)</sup> (Metanalysis)	CABG, Valve Surg Congenital Dis	Limb Ischaemia	Reduced Troponin in RIPC group
2012	D'Ascenzo <sup>(227)</sup> (Metanalysis)	CABG	RIPC	Reduced Troponin in RIPC group
2012	Hong <sup>(228)</sup>	Off-pump CABG	Lower limb 4 X 5 min	Reduced Troponin in RIPC group
2012	Lucchinetti <sup>(229)</sup>	On-pump CABG	Lower limb 4 x 5 min	No reduction in Trop in RIPC group
2011	Karuppasamy <sup>(230)</sup>	CABG, no valve	Upper limb 3 x 5 min	No significant difference in Trop, BNP, CKMB level
2010	Hong <sup>(231)</sup>	Off-pump CABG	Upper limb 4 x 5 min	No statistical difference in Troponin release
2010	Wagner <sup>(232)</sup>	CABG ±AVR	Upper limb 3 x 5 min	Late phase-RIPC can reduce injury

**Table 2- RIPC trials to date in adult cardiac surgery (Continued)**

Year	Author	Clinical Setting	Intervention	Outcome
2010	Ali <sup>(233)</sup>	CABG	Upper limb 3 x 5 min	Significant reduce in CKMB in RIPC group
2010	Thielmann <sup>(234)</sup>	CABG	Upper arm 3 x 5 min	Significant reduce in Trop in RIPC group
2010	Rahman <sup>(235)</sup>	CABG	Upper arm 3 x 5 min	No decrease in Troponin release
2009	Venugopal <sup>(236)</sup>	CABG	Upper limb 3 x 5 min	Reduced Troponin release in RIPC
2007	Hausenloy <sup>(237)</sup>	CABG	Upper limb 3 x 5 min	Reduced Troponin in RIPC group
2000	Gunaydin <sup>(205)</sup>	CABG	Upper limb  2 x3 min	Myocardium protected
1997	Jenkins	CABG	2 additional X 3 min myocardial ischaemia	Myocardium protected

## Mechanism of RIPC

Numerous extensive investigations have taken place over the last 20 years on RIPC, but its mechanism still remains unclear. Several hypotheses however have been proposed. Experimental studies suggest a neuro-hormonal pathway linking the remotely preconditioned organ to the heart where myocardial pro-survival signalling pathways are activated. <sup>(238, 239)</sup>

Some of the underlying mechanisms of conventional myocardial preconditioning and postconditioning are believed to also play a role in RIPC setting. <sup>(238, 240-243)</sup>

From the site of the remote stimulus, through humoral and neuronal pathways, remote ischaemic preconditioning activates several protective mechanisms in the target organ similar to those activated by local preconditioning. <sup>(244)</sup> They include the reperfusion-injury salvage kinase and survivor activating factor enhancement signalling pathways. <sup>(245)</sup>

Furthermore, RIPC modifies the systemic inflammatory response, <sup>(246, 247)</sup> prevents endothelial dysfunction and platelet activation <sup>(248)</sup> following ischaemia-reperfusion injury. Classic Ischaemic Preconditioning, Remote Ischaemic Preconditioning and Post Conditioning, share common signalling pathways, including the release of cardioprotective autocooids such as adenosine <sup>(249)</sup> and nitric oxide <sup>(250)</sup> and bradykinin which are responsible in triggering cardioprotection effect on endothelial function, <sup>(60, 251, 252)</sup> activation of the reperfusion injury salvage kinase (RISK) pathway, the inhibition of the mitochondrial permeability transition pore opening, <sup>(253, 254)</sup> mediators (protein kinase C activation, and end effectors), <sup>(197)</sup> activation of prosurvival kinases [PI3K-Akt, Erk1/2]. <sup>(255-258)</sup>

Hausenloy & Yellon <sup>(238)</sup> have simplified the RIPC mechanism as three inter-related events below  
(1)The application of brief episodes of ischaemia and reperfusion to the remote organ or tissue,

releases endogenous autocooids or factors which can protect the heart from subsequent injury.

(259, 260)

(2) The cardioprotective message/signal, which is conveyed from the remote organ or tissue to the myocardium. Two hypotheses are playing a role here: The cardioprotective signal either comprises a blood-borne factor that is carried from the remote organ or tissue to the heart and/or that it is the activation of a neural pathway which mediates the cardioprotective effect.

(240)

(3) The events occurring in the myocardium in response to the above relayed message, which confer the cardioprotective effect.

## **Neuro-Humoral Pathway**

The role of potential factors conveying messages between remote organs and the heart has been investigated extensively over the last few years. It is believed that two major relay mechanisms exist:

- 1) Humoral factor which releases in the distal organs and then travels to the heart;
- 2) Neural pathway between the remote organ and the myocardium.

RIPC induced by brief episodes of ischaemia and reperfusion applied to the limb requires the neural or the humoral pathways to limit myocardial infarct size.

Various endogenous autocooids are released in the remote organs. They either travel in the blood to the heart and effect directly or activate efferent neural pathways within the remote organ to confer protection.

The actual interplay between the neuronal system and humoral factor remain unknown.<sup>(261)</sup> Further study is required to elucidate the interplay between these two mechanistic pathways underlying cardioprotection.

## Humoral Factor in RIPC

The hypothesis that a blood borne humoral factor is playing an important role in conveying preconditioning signal from the remote organ to the heart and in mediating systemic spread of cardio-protection, was supported by an observation that showed protection could be transferred by injection of serum from a preconditioned rabbit to a rabbit which has not been preconditioned before undergoing induced myocardial infarction.<sup>(262)</sup>

Dickson et al demonstrated that blood taken from a preconditioned rabbit and transfused to a non-treated rabbit, reduced the infarct size by 69%. The presence of humoral factor in RIPC was later on strongly confirmed in a robust study performed by Konstantinov et al. and Kristiansen et al.<sup>(204, 263)</sup> RIPC using brief episodes of ischaemia reperfusion in a recipient pig was able to limit infarct size of the denervated donor heart.

More importantly, a period of reperfusion of the remote conditioned organ was required to achieve cardioprotection, suggesting that protective stimulus required wash-out of a protective blood-borne humoral factor generated in the conditioned site into the circulation.<sup>(190, 264)</sup>

The nature of the circulating substance is unknown and might vary with species or stimulus, but it could function through opioid, endocannabinoid, or angiotensin-1 receptors and other G-protein-coupled receptors. Patel et al. have shown that non-specific opioid receptor antagonist naloxone, abolishes the cardioprotective effect of RIPC in rats.<sup>(265)</sup>

The humoral hypothesis proposes that activation of receptors of adenosine<sup>(186, 190, 261)</sup> bradykinin<sup>(197)</sup> opioids<sup>(265, 267) (263)</sup> erythropoietin<sup>(268, 269)</sup> CB 2 endocannabinoid<sup>(270)</sup> angiotensin-I<sup>(194)</sup> and prostaglandin<sup>(266)</sup> receptors and the associated signalling pathways are implicated in mediating the protective effect of RIPC. However, whether they constitute the endogenous substances that are generated in the remote conditioned organ or tissue and being transported to the injured organ target through blood circulation remains unknown.<sup>(267)</sup>

The actual identity of circulating humoral factors remains unknown. But following numerous studies, we now know that the cardioprotective humoral factors, generated in response to RIPC of limb are likely to be hydrophobic and between 3.5-8 Da in size.<sup>(268-270)</sup>

## **Role of Neural Mechanism in RIPC**

Several experimental studies have implicated a neuronal pathway mediating the connection between the remote conditioned organ or tissue to the protected organ and tissue.<sup>(238)</sup> Neurogenic mechanisms have been explored using autonomic ganglionic blockade. In a rat myocardial infarction model, hexamethonium (a ganglion blocker) abolished RIPC induced cardioprotection which was achieved by mesenteric artery occlusion. It had no effect on myocardial ischaemic preconditioning. Cardioprotection was absent when mesenteric artery occlusion was sustained throughout the study, indicating that reperfusion in the small intestine was essential to activate the neurogenic pathway. gho<sup>(190)</sup>

According to Lim & Hausenloy<sup>(272)</sup>, the current understanding of the neuronal pathway involves the release of endogenous autocooids, including neuropeptides such as Calcitonin Gene Related Peptide (CGRP)<sup>(276, 277)</sup> adenosine DING<sup>(189, 196, 276, 274)</sup> and bradykinin<sup>(195)</sup>, from the remotely

conditioned organ or tissue which then activates local afferent nerves, which then stimulate efferent nerves that terminate at the remote organ and tissue to mediate protection.<sup>(186, 195)</sup>

The role of adenosine in RIPC was first investigated in the Yellon Lab in 1998 on rabbits.<sup>(189)</sup>

Cardioprotection was abolished in rabbits treated with adenosine antagonist 8-sulphophenyltheophylline (8-SPT).

Bradykinin and CGRP are other endogenous substances implicated in neural pathway. The role of bradykinin in RIPC was shown in rats by Schoemaker et al.<sup>(195)</sup> They showed that bradykinin blocker HOE140, abolished the cardioprotective effect, and injection of bradykinin into mesenteric artery was protective. This effect was blocked by hexamethonium, suggesting that bradykinin acts through neural afferent stimulation.

Recent research has emphasised the necessity of intact neural pathways to the organ or tissue receiving the preconditioning trigger.<sup>(279)</sup>

## **Systemic Inflammatory Response in RIPC**

In addition to release of neurogenic and circulating factors, the stimulus of transient limb ischaemia has other biological effects that might be relevant to its effectiveness. A third hypothesis proposes that transient ischaemia and reperfusion of an organ or tissue provokes a systemic protective response involving modulation of immune cells either at post-translational level or through transcriptional regulation.<sup>(280)</sup> In a study done by Kharbanda group on healthy volunteers, the stimulus suppressed expression of pro-inflammatory genes in circulating leucocytes within 15 minutes, and still further at 24 hours.<sup>(280)</sup> The activated gene transcription was anti-inflammatory and anti-apoptotic.

## Myocardial Mechanisms of Cardioprotection in RIPC

Once the cardioprotective signal reaches the myocardium from the remote organ, intracellular signal transduction mechanisms that are similar to those that participate in ischaemic preconditioning and postconditioning, get recruited within the cardiomyocytes. Hausenloy and Yellon<sup>(238)</sup> These include the ligand binding to G-protein cell surface coupled receptors, which then activate intracellular kinases such as PKC- $\epsilon$  and other signalling components such as reactive oxygen species (ROS), Nitric Oxide (NO) and the mitochondrial  $K_{ATP}$  channel which act the same way as described in classic preconditioning.<sup>(191)</sup> Preconditioning has a beneficial platelet inhibitory and antithrombotic effect, which might stabilise vulnerable plaques,<sup>(282)</sup> improve endothelial function, and reduce inflammation.<sup>(40)</sup>

### $K_{ATP}$ -Dependent Mechanism

Intermittent peripheral tissue ischaemia during coronary ischaemia reduces myocardial infarction through a  $K_{ATP}$ -dependent mechanism; first demonstration of remote ischaemic preconditioning.

$K_{ATP}$  is a clinical trigger in the cardioprotective phenomenon. Schmidt et al.<sup>(178)</sup> tested the hypothesis that short periods of limb ischaemia administered at the same time of established myocardial ischaemia, would reduce MI, and the term “remote per-conditioning” (Per-C) was proposed to emphasise that the stimulus is administered during coronary ischaemia. The role of the ATP-dependent ( $K_{ATP}$ ) channel in this form of myocardial protection was also tested in this study.<sup>(178)</sup> Opening of  $K_{ATP}$  channels, particularly the mitochondrial subtype, has been shown to be a pre-requisite for the induction of protection against ischaemic reperfusion injury by IPC,<sup>(283)</sup> RIPC,<sup>(189)</sup> and Postconditioning.<sup>(254)</sup>



## **Role of Nitric Oxide in RIPC**

Nitric oxide plays a critical role as a mediator of cardioprotection in the setting of both classical and delayed IPC.<sup>(171, 284)</sup> Tokuno et al.<sup>(192)</sup> have implicated inducible nitric oxide synthase (iNOS) activation as a trigger for delayed RIPC of the heart, using cerebral ischaemia as the preconditioning stimulus. They demonstrated that the bilateral occlusion of the internal carotid arteries to induce permanent cerebral ischaemia could reduce myocardial infarct size in murine hearts 24 hours later.

## **Role of Protein Kinase C**

It is well established that Protein Kinase C (PKC) plays a critical role as a mediator of the preconditioning signal in the setting of IPC with the PKC-1 isoform being the major cardioprotective isoform.<sup>(171)</sup> A few experimental studies have demonstrated the non-specific PKC blocker, chelerythrine, can abolish that cardioprotection.<sup>(197, 264)</sup> Bradykinin, which is also a well-known mediator, acts via activating PKC. It is now understood that PKC plays an important role in ROS production.

## **Role of Reactive Oxygen Species**

Oxidative stress appears to play a dual role in the setting of acute myocardial ischaemia-reperfusion injury. During ischaemia, anaerobic metabolism predominates and ATP production decreases. This ischaemia inevitably leads to cell death, unless blood flow is restored. Though

reperfusion with oxygenated blood is essential for any tissue salvage, the sudden influx of oxygen leads to the formation of reactive oxygen species. However, its beneficial signalling role is believed to mediate the cardioprotective effects elicited by both ischaemic preconditioning and postconditioning.<sup>(171)</sup>

A study by Weinbrenner et al.<sup>(285)</sup> suggests a possible beneficial signalling role for reactive oxygen species (ROS) in the setting of RIPC. They discovered that a free radical scavenger was able to abolish the protection elicited by RIPC. Whether the free radicals are generated in the preconditioned organ or tissue or in the myocardium is currently unclear and requires further examination.

## **Mitochondrial Permeability Transition Pore**

Mitochondrial Permeability Transition Pore (mPTP), another probable regulator of preconditioning, is a large-conductance mega channel that is closed under physiological conditions.<sup>(286)</sup> A key event in cell death is mitochondrial permeability transition, a phenomenon that occurs when the mPTP becomes permeable to molecules of 1500 kDa or smaller. This leads to a rapid influx of small molecules, mitochondrial swelling, and subsequent cell death.<sup>(286, 287)</sup>

Its detrimental role in the first few minutes of myocardial reperfusion is a mediator of lethal reperfusion injury. Veighey<sup>(257, 288)</sup> It mediates cell death by uncoupling oxidative phosphorylation leading to ATP depletion and by inducing mitochondrial swelling.<sup>(286)</sup> Preventing its opening at the time of myocardial reperfusion, exerts powerful cardioprotection, a mechanism which is believed to underpin the endogenous cardioprotective phenomena of IPC and post-conditioning.

The mitochondria are a major site of cellular energy and ATP production. Their role in preconditioning was supported by the observation that glyburide—a K-ATP channel blocker—blunts the conditioning effect.<sup>(289)</sup> During ischaemia–reperfusion there is high concentrations of Ca<sup>2+</sup>, ROS, inorganic phosphate, nitric oxide and a reduction of the inner membrane potential, and all these conditions favour opening of the pore.<sup>(236)</sup> Regulation of K<sub>ATP</sub> and mPTP provides protection from ischaemia or generate intermediates such as free radicals that play important role in myocardial protection.<sup>(290)</sup> The mPTP is the most important end-effector of cardiac conditioning. It has not been explored yet how the various cellular components within the myocardium contribute to the conditioning phenomenon. Endothelium has essential role in the regulation of vascular tone as well as thrombosis, platelet and leukocyte function; hence it may have a significant role. Extracellular receptors offer considerable opportunity for inter and intracellular interaction, and the study of these may offer new insights into mechanisms underlying conditioning the heart.

## Delayed RIPC

Delayed RIPC is one of the novel concepts of remote ischaemic conditioning. Similar to classic conditioning, delayed RIPC has also got 2 phases: Early RIPC results in acute protection through post-translational protein modification and delayed RIPC providing second phase of prolonged protection against ischaemic injury. An elegant temporal characteristic study by Moses et al. <sup>(291)</sup> has demonstrated that the therapeutic time window of delayed RIPC is similar to conventional IPC, i.e., from 24 hrs and lasting for up to 72 hrs.

## Ischaemic Postconditioning

Ischaemic preconditioning (IPOC) has to be applied before the onset of ischaemia. This requirement has restricted the clinical application of preconditioning to elective procedures when the ischaemic episode can be anticipated. Interestingly if a similar intervention applies at the time of perfusion, heart can still be protected from ischaemic reperfusion injury. This is called ischaemic postconditioning. In this setting the protection against ischaemia/reperfusion takes place after the harmful injury is induced. Brief episodes of ischaemia immediately following reperfusion after a prolonged ischaemic insult proved to be beneficial in animal models <sup>(266, 292-294)</sup> as well as in humans. <sup>(295)</sup> IPOC may protect the myocardium via multiple mechanisms, such as the reperfusion injury salvage kinase pathway, the survivor activating factor enhancement pathway and the activation of protein kinase G. These pathways may all eventually result in prevention of opening of the mitochondrial permeability transition pore. The term ischaemic postconditioning was first used in 1996 by Na and co-workers <sup>(296)</sup> who demonstrated in cats that applying intermittent reperfusion by method of premature ventricular contractions could reduce reperfusion-induced ventricular fibrillation following myocardial ischaemia. In 2005, Staat et al. showed that repeated episodes of ischaemia/reperfusion, by inflating the angioplasty balloon four times for 1 min following direct stenting of the occluded culprit coronary artery was associated with reduction of CK release, an indicator of infarct size, by 36%.<sup>(297)</sup>

In one large study, one hundred and eighteen patients with STEMI underwent PPCI with or without postconditioning by four 30-seconds cycles of ischaemia–reperfusion immediately before PPCI. Postconditioning was associated with a decrease of 18% ( $P=0.037$ ) in infarct size, measured by CMR at 3 months.<sup>(298)</sup> Another recently published trial did not confirm this effect

though.<sup>(299)</sup> Furthermore, a large-scale trial of 700 patients admitted with STEMI randomised to either standard PPCI or PPCI followed by postconditioning, failed to show any effect on myocardial reperfusion and clinical endpoints.<sup>(300)</sup> When the concept of postconditioning was first described, the mechanism of protection was initially attributed to reduction of oxidative stress, decreasing intracellular calcium overload, improving endothelial function and reducing inflammation.<sup>(292)</sup> Since then, mechanisms underlying the cardioprotective effects of IPOC have been the subject of intense investigation, including mPTP opening during the first minutes of reperfusion, which is hypothesised by Yellon & Hausenloy in 2007.<sup>(67)</sup> This crucial inhibitory event is mediated by activation of survival signalling pathways (reperfusion injury salvage kinases, RISKs), which in turn are triggered by the activation of specific cell-surface receptors.<sup>(301)</sup> Similar to preconditioning, the postconditioning stimulus also appears to activate G protein-coupled receptors (GPCRs) by endogenous ligands, such as adenosine, opioids, and bradykinin, providing the initial trigger for recruitment of survival signalling pathways. The adenosine receptor was the first GPCR to be linked with IPOC. Thus, the reduction in myocardial infarct size elicited by IPOC was abolished in the presence of 8-p- (sulphophenyl) theophylline, a non-specific adenosine receptor blocker.<sup>(302)</sup>

A number of different signalling pathways have been reported to mediate the protective effects of postconditioning, most notably the RISK pathway. The RISK pathway describes a group of survival protein kinases (ERK1/2 and PI3K/ Akt), which when activated at the time of reperfusion confer powerful cardioprotection. Pharmacological agents, including growth factors, GPCR agonists, cytokines, natriuretic peptide, adipocytokines and statins, administered at the immediate onset of reperfusion activate either ERK1/2 or PI3K/Akt and accordingly reduce myocardial infarct size (pharmacological postconditioning) in animal models of acute MI.<sup>(301)</sup>

## Preconditioning in other Organs than Heart

As discussed above, RIPC was originally designed as a therapeutic strategy for protecting the myocardium against the ischaemic reperfusion injury. Later on however, a number of experimental and clinical studies demonstrated that RIPC may protect kidneys,<sup>(199, 293, 304)</sup> lungs,<sup>(203, 235, 305)</sup> liver,<sup>(306-308)</sup> ovaries,<sup>(309)</sup> intestine,<sup>(309, 310)</sup> stomach<sup>(271)</sup> and pancreas.<sup>(312)</sup> The cerebral protective roles of limb RIPC in the animal models of transient focal cerebral ischaemia or whole cerebral ischaemia is now confirmed.<sup>(313)</sup>

## Factors that Blunt the Protective Effect of Preconditioning

The effect of age and other cardiovascular risk factors on pre and postconditioning is of clinical significance because a large proportion of patients with coronary artery disease who might benefit from conditioning are elderly or have metabolic conditions that may attenuate the protective effect.<sup>(314)</sup> The aged heart is accompanied by an impaired ability to translate both endogenous stress signals and pharmacological activators into biochemical steps necessary for induction of the cardioprotective response.<sup>(315)</sup>

These factors are explained in details in the last 2 chapters of this thesis and listed in the table

1.4.

**Table 1.4**

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### Factors that are believed to attenuate preconditioning and postconditioning effect

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Age<sup>(316-321)</sup>

Diabetes<sup>(322, 323)</sup>

Glibenclamide<sup>(324)</sup>

Smoking<sup>(325)</sup>

Hyperlipidaemia<sup>(326, 327)</sup>

Hypertension<sup>(327)</sup>

Obesity<sup>(328)</sup>

Antecedent angina<sup>(177)</sup>

Direct preconditioning effect from coronary balloon inflation<sup>(292)</sup>

GTN<sup>(185)</sup>

Nicorandil<sup>(314)</sup>

Statins<sup>(329)</sup>



## PCI related Contrast-Induced Kidney Injury

Contrast-induced acute kidney injury (CI-AKI) or contrast-induced nephropathy (CIN), which is an impairment of renal function resulting from administration of contrast media in the absence of an alternative etiology,<sup>(330, 331)</sup> is a prevalent but under-diagnosed complication of diagnostic coronary angiography and PCI. CI-AKI is defined as either an absolute increase in serum creatinine (Cr) concentration of 44.2 µmol/L (or 0.5 mg/dl) or a 25% relative increase of Cr from baseline.<sup>(332, 333)</sup>

CI-AKI typically manifests within 3 days of contrast media administration, peaks within 3 to 5 days, and resolves within 10 to 21 days.<sup>(334)</sup> Although often a transient injury, it can be associated with increased in-hospital morbidity and mortality.

The importance of this complication is being increasingly recognised. Several recent North American and European epidemiological studies have shown that the incidence of acute kidney injury (AKI) is increasing at an alarming rate.<sup>(335)</sup> CI-AKI has been reported to be the third most common cause of hospital-acquired renal failure.<sup>(336,337)</sup> The incidence of acute renal insufficiency after PCI ranges from 2.0% in those patients with normal baseline renal function to as high as 20–30% in those patients with a baseline creatinine greater than 176 µmol/L (or 2.0 mg/dl) prior to PCI.<sup>(338, 330, 331)</sup> Nash et al<sup>(336)</sup> reported that 11% of hospital-acquired renal insufficiency cases are due to contrast media, with coronary angiograms and PCI being the leading cause. The incidence of AKI requiring dialysis following PCI, however, is fortunately rare, and is less than 1%.<sup>(339)</sup>

Pre-existing chronic kidney disease (CKD) is the most important risk factor for developing CI-AKI post-PCI. Diabetes mellitus has been identified as another important risk factor. In patients with

diabetes mellitus and CKD, the incidence of CI-AKI increases an additional two-fold, and can reach as much as 33%.<sup>(340,341)</sup> There is no definitive evidence that CI-AKI correlates with the duration of diabetes or suboptimal glycaemic control, but tight glycaemic control should be achieved before contrast media exposure.<sup>(342,343)</sup>

The incidence of CI-AKI is also significantly higher in patients with several other comorbidities, including congestive heart failure, hypotension, hypertension, pre-procedure shock, recent myocardial infarction (MI), old age and female gender.<sup>(344, 345)</sup>

The apparently increased prevalence of CI-AKI in elderly patients is likely to be multifactorial in origin and may be attributable to the presence of large or small vessel renal arteriosclerosis, underestimation of background CKD in this group, impaired cardiovascular performance (and hence renal oxygen delivery) and reduced regenerative capacity of the renal parenchyma in the face of acute injury.<sup>(338, 342)</sup>

The incidence of CI-AKI may also be increased by concomitant use of nephrotoxic agents such as non-steroidal anti-inflammatory drugs and angiotensin-converting enzyme inhibitors, which are used in more than 60% of cardiac patients.<sup>(346)</sup>

The risk of CI-AKI increases if the PCI is undertaken in the context of reduced effective circulatory volume. This may be due to hypovolemia (including overdiuresis), liver failure or cardiac failure. Sepsis, hypercalcaemia and rhabdomyolysis are further risk factors for developing CI-AKI.<sup>(343)</sup>

The risk of CI-AKI is significantly higher among patients with acute MI undergoing PCI than among stable CHD patients undergoing elective PCI.<sup>(347)</sup> The most likely contributing factors for CI-AKI in this context are impaired systemic perfusion caused by left ventricular dysfunction, the need for the administration of large volumes of contrast medium, and the lack of sufficient time to perform renal prophylactic therapies prior to contrast medium exposure.

In these high-risk patients, the incidence of CI-AKI has been estimated to range from 20-50% depending on the intervention and criteria used to diagnose AKI. <sup>(340-348,349)</sup> Even a mildly increased serum creatinine post-PCI is a predictor of worse clinical outcome. A serum creatinine level of only 115  $\mu\text{mol/L}$  (or 1.3 mg/dL), which in most patients indicates a reduction in renal function of about 50%, is associated with a two-fold increase in total mortality and a 10% reduction in cumulative survival over three years. <sup>(350)</sup> Despite technical advances in PCI, there is increasing recognition that CI-AKI can have serious short term and long-term consequences such as in-hospital death, long term mortality, progression of chronic kidney disease and increased health care expenditure. <sup>(332,350)</sup>

## **Pathophysiology of CI-AKI**

The pathophysiology of CI-AKI is still ill-defined and poorly understood. Implicated mechanisms include changes in the renal circulation leading to ischaemic damage to the renal medulla and the production of oxygen free radicals inducing tubular epithelial damage. <sup>(330,331)</sup> Haemodynamic instability with reduced effective arterial volume during the procedure, microemboli to the kidney and concomitant drug toxicity are other important factors that may be responsible for CI-AKI following PCI. <sup>(352)</sup>

Experimental findings indicate that contrast media administration rapidly induces a renal vasoconstrictive response. This has been ascribed to a number of different mediators, such as the renin-angiotensin system, changes in the intracellular calcium concentration of smooth muscle cells, adenosine and endothelin. <sup>(352,353)</sup>

## Contrast Osmolarity

Contrast nephrotoxicity and the risk of developing CI-AKI are related to the osmolarity of the contrast media. This reflects the total particle concentration of the solution (the number of molecules dissolved in a specific volume).<sup>(354)</sup> It is widely accepted that contrasts with a high osmolarity are associated with the greatest risk of developing CI-AKI. As a consequence, the osmolarity of available contrast media has been gradually decreasing to physiological levels over the last 40 years. In the 1950s, only high-osmolar contrast media (e.g., diatrizoate) with osmolality five to eight times of plasma was available. In the 1990s, isosmolar contrast media (e.g., iodixanol) were developed and are now widely in use.<sup>(355)</sup>

## Emerging biomarkers for early detection of AKI

The substantial lag time between renal injury and the consequent rise in serum Cr has disadvantages. Interventions to mitigate or reverse renal injury may be substantially delayed, while prognostic staging, and the planning of care, is dependent on serum Cr measurements extending to several days, which do present logistical difficulties. The emergence of novel biomarkers offers an opportunity to diagnose AKI at an earlier stage, which can differentiate between structural and functional AKI, and predict the outcome of established AKI.<sup>(356)</sup> The most promising renal biomarkers include plasma and urine neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), Clusterin, Cystatin C, and interleukin 18 (IL-18).<sup>(357-359)</sup> In a recent meta-analysis, it was confirmed that NGAL is a valuable renal biomarker in all settings of AKI investigated.<sup>(360)</sup> The role for biomarkers in the early detection and staging of CI-AKI is the subject of ongoing clinical research.

Currently, the diagnosis of CI-AKI is dependent on determining changes in serum Creatinine level. However, in CI-AKI, serum Cr values do not rise immediately after the contrast insult. Although there are clearly limitations in the effectiveness of using serum Cr for the rapid detection of contrast mediated renal injury, rises in serum Cr in hospitalised patients have been strongly associated with outcomes, including length of stay, mortality and healthcare expenditure. There is, however, growing interest in the use of more novel biomarkers that might provide a more rapid diagnosis of CI-AKI, particularly if their use might influence pre-emptive measures or subsequent monitoring.

## **Neutrophil Gelatinase-Associated Lipocalin (NGAL)**

There is an urgent need for early predictive biomarkers of both acute kidney injury (AKI, previously referred to as acute renal failure) and chronic kidney disease (CKD). In both situations, early intervention can significantly improve the prognosis. However, currently available biomarkers such as serum creatinine concentrations lack enough accuracy and details, and their delayed response delays potentially effective therapies in a timely manner. Prompt diagnosis and management of AKI can minimise the adverse effects post PCI. The development of novel biomarkers such as (NGAL) is promising and may enable more rapid detection of CI-AKI. NGAL is an emerging biomarker of acute kidney injury, with equivalent increases of both urinary and plasma NGAL within 6 h of renal the insult- a time course that, should it be reciprocated in angiographic patients- may offer earlier opportunities for mitigating renal injury and for assessing prognosis. <sup>(372)</sup> Several investigators have examined the role of NGAL as a predictive biomarker of nephrotoxicity following contrast administration. <sup>(373)</sup>

## Preventing CI-AKI post-PCI

It is important that patients undergoing PCI have an appropriate risk assessment for PCI-AKI. Precautionary measures before, during, and after the use of contrast media that reduce the incidence of CI-AKI, such as discontinuation of nephrotoxic medications, and the use of appropriate volumes and types of contrast media, should be considered in all patients with renal insufficiency or with other risk factors for CI-AKI. Low osmolar non-ionic contrast is the contrast of choice for all patients at high-risk for post-PCI renal insufficiency.

Pre-PCI hydration remains the most effective approach to preventing CI-AKI. Risk assessment and the institution of preventive therapy such as preventing dehydration is essential.

One of the mechanisms hypothesised to be responsible for CI-AKI development is the development of oxidative stress with the intra-renal accumulation of reactive oxygen species. Alkalinisation of tubular fluid has been shown to diminish the production of free oxygen radicals.

<sup>(361)</sup> This has led to the investigation of sodium bicarbonate as a CI-AKI prophylactic therapy. Despite the fact that pre-treatment with sodium bicarbonate is more protective than sodium chloride in animal models of acute ischaemic renal failure, <sup>(362)</sup> the results of studies in man have been conflicting. <sup>(363-365)</sup>

To evaluate the available controversial data and to assess the effectiveness of Normal Saline versus Sodium Bicarbonate infusion a few meta-analyses have been performed. Meier et al <sup>(366)</sup> performed a meta-analysis of 17 randomised controlled trials including 2,633 patients. This suggested a significant benefit with the use of NaHCO<sub>3</sub>-based hydration for prophylaxis of CI-AKI. However, some other trials have failed to find any benefit in hydration with Sodium Bicarbonate.

<sup>(367-370)</sup>

The antioxidant N-acetylcysteine (NAC) has been suggested as a therapy to attenuate the risk of developing CI-AKI by scavenging oxygen free radicals generated as a result of renal tubular toxic damage. However, there has been ongoing debate over whether NAC is effective in preventing CI-AKI. The current literature suggests that its role as an adjunct to saline hydration in patients with mild to moderate renal insufficiency is limited. In the largest randomized study thus far assessing the efficacy of NAC for preventing CI-AKI, intravenous NAC (500 mg) did not provide renal protection in patients with impaired renal function compared with placebo.<sup>(371)</sup> There may be a role for this agent where complete hydration is not possible (e.g. emergency coronary angiography or symptomatic congestive heart failure), or in patients with more severe renal dysfunction (serum Cr >2.5 mg/dl),(70) however this requires further investigation.

Based on multiple studies, it is a common practice in most of UK hospitals to continue ACE-Is in patients with mild to moderate renal insufficiency who are undergoing PCI.

## **Protective role of RIPC in CI-AKI**

Current preventative interventions are largely centred on the avoidance of dehydration, and pre-treatment with N-acetyl cysteine and/or sodium bicarbonate, withdrawal of nephrotoxic agents and minimisation of contrast load but none of the above measurements can be entirely protective. The efficacy is limited in high risk patients and CI-AKI remains significant post PCI.

Therefore there is a potential role for novel therapies that can improve the outcome and prevent CI-AKI in patients undergoing PCI. In this respect, the safe and low-cost therapeutic intervention of remote ischaemic preconditioning is a potential strategy for preventing CI-AKI. Proof-of-concept clinical studies have shown that RIPC using transient ischaemia and reperfusion

of the lower limb can preserve kidney function in patients undergoing elective endovascular, <sup>(374)</sup> open surgical repair of an abdominal aortic aneurysm, <sup>(375)</sup> and coronary artery bypass graft surgery. <sup>(234)</sup> It has recently been demonstrated in a proof-of-concept clinical study that RIPC could reduce the incidence of CI-AKI in PCI patients. <sup>(376)</sup> Large randomised controlled trials are now required to confirm the efficacy of RIPC in CI-AKI and investigate whether clinical outcomes can be improved.

Therefore, in the ERIC- PCI study, we hypothesised that the safe and low-cost therapeutic intervention of RIPC which refers to a powerful endogenous protective phenomenon whereby brief episodes of non-lethal ischaemia and reperfusion to one organ confers protection against a sustained lethal episode of ischaemia and reperfusion in another organ, is a potential strategy for preventing CI-AKI.



# **CHAPTER 2**

## **AIMS and OBJECTIVES**

Despite significant advances in revascularisation techniques, PCI-related myocardial injury is still common, and leads to worse prognosis even after an angiographically successful procedure. PCI-related myocardial injury results from procedural complications of PCI, such as distal embolisation, side branch occlusion, coronary dissection, and disruption of collateral flow. Although not entirely agreed by all scientists, reperfusion injury might also play a role in pathogenesis of PCI-related myocardial injury.

Patients undergoing PCI for complex lesions are at increased risk of procedural-related injury and require better cardioprotection. During complex coronary intervention, a higher amount of contrast media might be used which increases the risk of contrast-induced acute kidney injury (CI-AKI).

The role of RIPC in decreasing the amount of PCI-related myocardial and renal damage is the focus of this thesis.

We therefore evaluated the cardio-protective effect of RIPC in the setting of complex PCI.

## **Overall Hypothesis**

RIPC using transient limb ischaemia and reperfusion will reduce procedural-related myocardial injury in patients undergoing complex PCI.

## **Primary Objective**

To investigate in patients undergoing complex PCI whether RIPC using transient limb ischaemia and reperfusion will reduce the incidence and extent of PCI-related myocardial injury as assessed by serum cardiac biomarkers.

## Secondary Objectives

1. To investigate in patients undergoing complex PCI whether RIPC using transient limb ischaemia and reperfusion will result in less chest pain and fewer ECG changes of myocardial ischaemia during the PCI procedure.
2. To investigate in patients undergoing complex PCI whether RIPC using transient limb ischaemia and reperfusion will reduce PCI-related myocardial injury as evidenced by a reduction in the incidence and extent of myocardial oedema and necrosis on cardiac MRI.
3. To investigate in patients undergoing complex PCI whether RIPC using transient limb ischaemia and reperfusion will reduce the incidence and severity of contrast-induced acute kidney injury (CI-AKI)
4. To investigate in patients undergoing complex PCI whether RIPC using transient limb ischaemia and reperfusion will reduce the rates of coronary revascularisation, re-infarction, and cardiovascular death at 30 days.

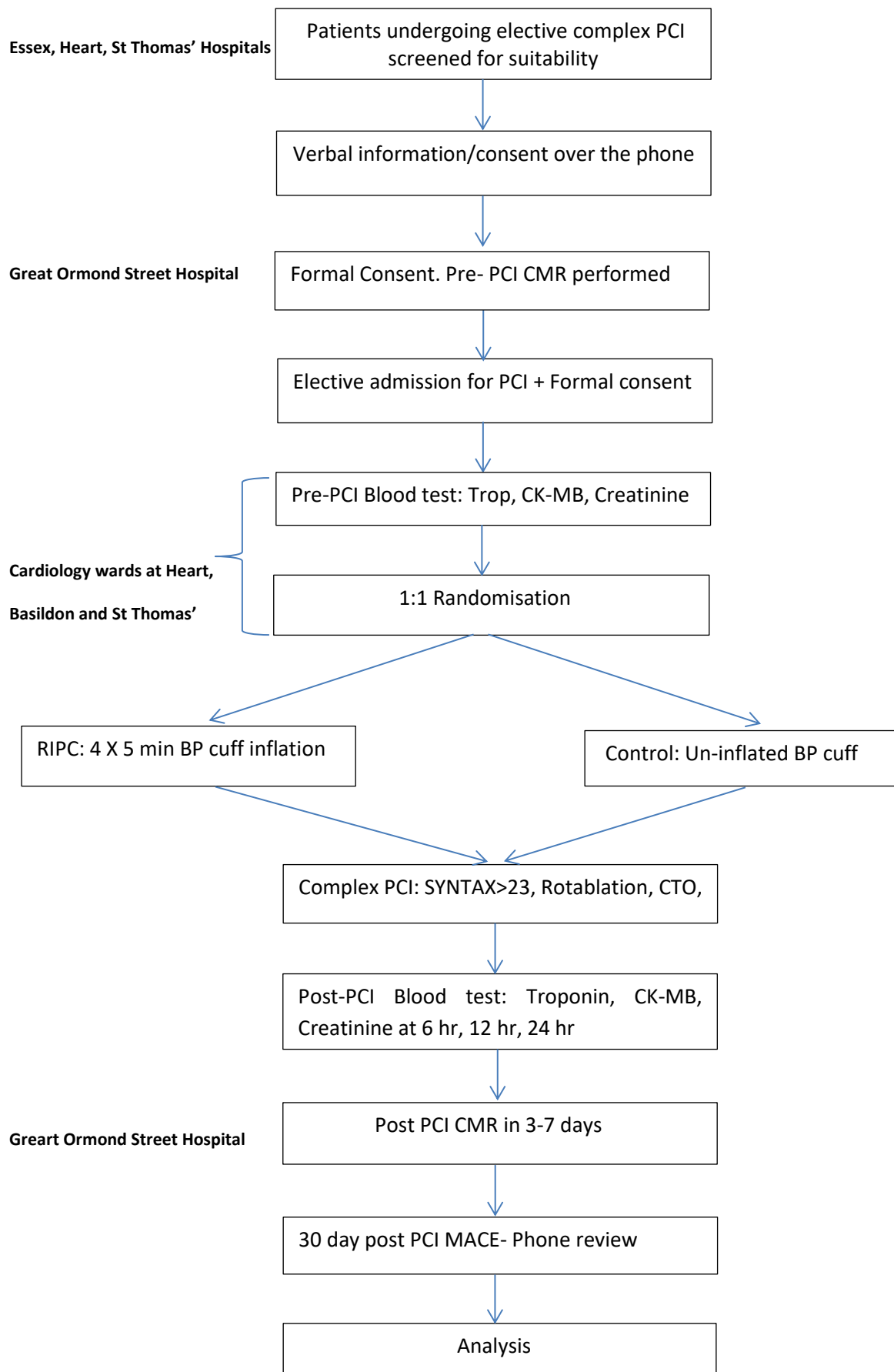
# **CHAPTER 3**

## **METHODS**

## Overview of the ERIC-PCI Study

The ERIC-PCI trial was conducted in three cardiac centres (The Essex Cardiothoracic Centre, The Heart Hospital and St Thomas' Hospital) as a single-blinded randomised-controlled trial. The primary objective of this study was to investigate the efficacy of RIPC using transient limb ischaemia and reperfusion, in reducing the incidence and extent of PCI-related myocardial injury in patients undergoing complex PCI. This potential effect was mainly assessed by measurement of two serum cardiac biomarkers; Troponin and CK-MB at time intervals zero (prior to RIPC or control intervention), 6, 12 and 24 hours post PCI. Patients awaiting elective complex PCI were randomly assigned in a 1:1 ratio to receive either the RIPC (intermittent arm ischaemia and reperfusion through four cycles of 5-minutes inflation and 5-minutes deflation of a blood-pressure cuff placed on the upper arm) or control protocols. In the control treatment arm, a standard un-inflated blood pressure cuff was placed on the upper arm for 40 minutes.

## ERIC-PCI trial overall patient pathway



## **Ethical, Research and Development (R&D) Approval**

The investigator ensured that this study was conducted in full conformity with the current revision of the Declaration of Helsinki, the principles of Good Clinical Practice and the Research Governance Framework. The protocol of this study was written in accordance with the International Conference on Harmonisation- Good clinical practice (ICH-GCP) guidance. The protocol contains multiple sub-studies for assessment of ischaemic preconditioning in different settings. The sub-study 5 of this protocol (the ERIC-PCI study) focuses on the effect of remote ischaemic preconditioning in complex PCI. The study was approved by the joint University College London (UCL)/ University College London Hospitals (UCLH) committees for the ethics of human research (REC 05/Q0502/102). The application form was submitted to the National Research Ethics Services (NRES) Committee London – Bentham, where the study protocol, patient information sheet (PIS), consent form and information letter to the general practitioners were approved. Following the favourable response of the NRES committee-London Bentham', separate applications were made to the departments of research and development (R&D) at the University College London and Basildon Hospitals, which were our two original recruitment centres. In order to increase patient recruitment, the third centre - Guy's and St Thomas' Hospitals NHS Trust- was invited to participate in this trial.

After initial approval, a request for a major amendment was made to the NRES committee as we also wished to assess the effect of RIPC on contrast- induced acute kidney injury (CI-AKI). The necessary major amendments to the protocol were made and submitted alongside updated versions of the patient information sheet, consent form and GP letter for a second favourable opinion of this study. Following approval of the new protocol and acceptance by the R&D departments, the logistics were carefully arranged and recruitment started in multiple centres.

The NRES Committee London – Bentham was also informed of any necessary minor amendments.

## **Patient Selection**

### **Inclusion Criteria**

1. Age > 18 years
2. SYNTAX score  $\geq$  23\*
3. Patient undergoing complex PCI defined as:

PCI to vein graft(s)

Rotational atherectomy assisted PCI

Laser assisted PCI

Chronic Total Occlusion (CTO) PCI

\*Syntax score is an angiographic grading tool and is calculated from the following angiographic variables: 1) Dominance 2) Number of lesions 3) Segments involved per lesion 4) Total occlusion 5) Trifurcation 6) Bifurcation 7) Aorto-ostial lesion 8) Severe tortuosity 9) length > 20 mm 10) Heavy calcification 11) Thrombus 12) Diffuse disease/ small vessels.

### **Exclusion Criteria:**

1. Inability to consent
2. Pregnancy
3. Participation in another trial
4. Peripheral vascular disease/contraindication to balloon cuff inflation
5. Recent Acute Coronary Syndrome (ACS) with raised Troponin



6. Peripheral vascular disease affecting the upper limbs
7. Standard contraindications to cardiac MRI scanning (Pacemaker or Implantable Cardioverter Defibrillator (ICD); Metal implants; significant renal impairment (e GFR <30); Claustrophobia)
8. Patients taking either glibenclamide\* or nicorandil\* were asked to withhold these medications for 48 hours

\*Nicorandil and glibenclamide can mimic ischaemic preconditioning. As per Yellon's laboratory report, nicorandil can mimic the protection of ischaemic preconditioning.<sup>(156)</sup> As well as dilating epicardial coronary arteries, nicorandil is involved in opening adenosine triphosphate-dependent potassium ( $K_{ATP}$ ) channels in ischaemic cardiomyocytes.<sup>(377)</sup> As the protection induced by preconditioning can be abolished by glibenclamide, patients were asked to stop taking this medication prior to the planned PCI date.<sup>(378)</sup>

## **Recruitment Procedure**

Elective patients undergoing complex PCI were recruited in a consecutive manner. In order to identify eligible patients, elective PCI lists at the three centres were screened by the researcher for suitability. Eligible patients at The Heart and St Thomas' hospitals were invited to participate in this trial via telephone. Following an initial explanation, verbal agreement to take part in this study was initially obtained over the phone. If participants agreed to undergo cardiac MRI scan, they were met first at the CMR department at Great Ormond Street Hospital for Children where the patient information sheet (PIS) was provided and formal written consent for the ERIC-PCI

trial was obtained. Following the pre-PCI Cardiac MRI, the second visit took place on the elective PCI day when randomisation and the study protocol were administered prior to PCI.

At The Essex Cardiothoracic Centre, eligible patients were approached in the PCI pre-assessment clinic and were provided with a patient information sheet. Formal written consent was obtained either at the CMR centre or on the cardiac ward on the day of admission for elective PCI. Following randomisation, patients received either the 'RIPC' protocol by means of intermittent blood pressure cuff inflation and deflation on the upper arm or the 'control' protocol. In patients randomised to the control treatment arm, a standard un-inflated blood pressure cuff was placed on the upper arm for 40 minutes. The operating interventionalist and the cardiac catheterisation laboratory staff were blinded to the treatment allocation. Post-PCI blood samples were collected 6 hours, 12 hours and 24 hours later. In patients who had a successful angioplasty and had CMR before PCI, post-PCI CMR was performed within the first 7 days after the procedure.

## **Consent Procedure**

Eligible patients from The Heart and St Thomas' hospitals were invited to participate in this trial by telephone. At The Essex Cardiothoracic Centre, eligible patients were directly approached in the PCI pre-assessment clinic. Following verbal consent over the phone, patients who agreed to undergo CMR scan -and did not have any contraindications -were asked to attend an appointment at Great Ormond Street Hospital for a pre-PCI MRI scan. Transport was arranged by The Hatter Cardiovascular Institute, UCL. Upon arrival, informed consent was obtained following extensive discussion of the risks and benefits of the trial as well as going through the patient

information sheet (PIS). On the procedure day, the recruitment process was explained in detail again and the PIS form was provided to patients who had not previously received it. Patients had sufficient time to read the patient information sheet and ask questions. Formal written consent was obtained using the latest appropriate version of the consent form. The original consent form was filed in the research folder. A copy of the consent form was given to the participants, and one copy was filed in the medical notes. The subjects could withdraw consent at any time throughout the course of the study. The rights and welfare of the subjects was protected by emphasising to them that the quality of their medical care would not have been adversely affected if they declined to participate in this recruitment.

## **Randomisation and Treatment Allocation**

On the PCI procedure day, formal informed consent was obtained on the cardiac ward if not already done so and patients were randomly assigned to the Remote Ischaemic Preconditioning or the control groups. For this purpose, a computer-generated randomisation sequence was used. Randomisation cards were sequentially numbered and kept in the sealed envelopes (SNOSE). The sealed envelopes were opened in presence of the patients. Study group randomisation, treatment allocation and delivery of RIPC were all performed by the unblinded researcher (lead investigator). The PCI operator, catheter laboratory staff and cardiac unit staff were all blinded to the treatment allocation.

## **Study Protocol**

All patients received the RIPC or control intervention within 3 hours of the planned PCI. According to the protocol, if the PCI was delayed for more than three hours, the intervention would be repeated prior to PCI. In the RIPC treatment group, a standard BP cuff was placed on the upper arm (right arm whenever possible) and inflated to 200mmHg and left inflated for 5 minutes at this pressure. The cuff was then completely deflated and left for 5 minutes at 0 mmHg. This cycle was repeated 4 times, so that the total duration of the intervention was 40 minutes. In patients randomised to the control treatment arm, a standard un-inflated BP cuff was placed on the upper arm for 40 minutes. The delivery of the RIPC or control interventions neither delayed nor overlapped the planned PCI procedure.

## **Percutaneous Coronary Intervention**

The decision on the choice of arterial access, revascularisation method, lesion(s) to be treated, the choice of wires/balloon/stents and medication use during PCI, were all based on the clinical judgment of the operating consultant interventionalist, and was not influenced by the research protocol. All patients received loading dose of dual antiplatelets (aspirin and clopidogrel) if they were not already on long term treatment. Intra coronary heparin was injected to all patients at a dose of 50-100 IU/Kg . As per decision of the operating interventionalist, intra coronary nitrate, adenosine or verapamil was administered as required. Detailed interventional events and parameters were recorded during the procedure. This included chest pain, ECG changes or any complications.

## **Primary Endpoint-Serum Cardiac Biomarkers**

The primary objective of this study was to investigate the efficacy of RIPC- using transient limb ischaemia and reperfusion- in reducing the incidence and extent of PCI-related myocardial injury in patients undergoing complex PCI. During PCI, passage of wire and balloon inflation, in another terms, instrumentation of the heart, inevitably results in myocardial injury with or without necrosis, whether or not accompanied by ST segment –T wave changes in ECG. Embolisation of intracoronary thrombus or atherosclerotic particulate debris induce inflammation of the myocardium surrounding islets of myocardial necrosis. <sup>(7)</sup> The occurrence of procedure-related cell necrosis can be detected by measurement of cardiac biomarkers Troponin and CK-MB, before and immediately after the procedure, and again at 6 to 12 and 18 to 24 hours later.

Cardiac troponin is the component of contractile structure of myocardial cells and is expressed almost exclusively in the heart with high specificity and sensitivity. Currently, Troponin is the only biomarker recommended by European and American guidelines for diagnosis of myocardial injury. Elevations of biomarkers above the 99th percentile upper reference limit (URL), assuming a normal baseline troponin value, are indicative of post-procedural myocardial injury. The development of more sensitive Troponin assays and precise imaging techniques has allowed detection of ever smaller amounts of myocardial necrosis/infarction. But this can have major psychological and legal implications. Therefore following Second Global MI Task Force, leading to the Universal Definition of Myocardial Infarction Consensus Document in 2007, which arbitrarily suggested to designate increases more than three times the 99<sup>th</sup> percentile URL, as PCI-related myocardial infarction, the 3<sup>rd</sup> universal definition for MI was published in 2012. According to this definition, PCI-related or Type 4a myocardial injury is defined ( still arbitrarily though) as elevations of cTn more than 5 times the 99th percentile upper reference limit (URL) occurring

within 48 hours of the procedure in addition to a) evidence of prolonged ischaemia (more than 20 min) as demonstrated by prolonged chest pain, or b) ischaemic ST changes or new pathological Q waves, or c) angiographic evidence of a flow limiting complication, such as loss of patency of a side branch, persistent slow-flow or no-reflow, embolisation or d) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.<sup>(3)</sup>

In the ERIC-PCI trial, due to logistic issues, assessing the cardiac enzymes 48 hours post PCI was not possible therefore elevation of Troponin and CK-MB levels occurring within 24 hours of the procedure was considered the primary endpoint. Levels above the baseline (zero-hour) and not the 99<sup>th</sup> percentile URL, were considered elevated. Blood samples were collected for hsTroponin T, CK and CK-MB levels at zero hours (prior to the randomisation protocol) and at 6, 12 and 24 hours post PCI. This was in addition to evidence of prolonged chest pain (more than 20 minutes), or ischaemic ST changes or new pathological Q waves, angiographic evidence of a flow limiting complication, imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

Detection of cardiac Troponin was based on an electrochemiluminescence immunoassay using a Tris (bipyridyl)-ruthenium (II) complex as a label.<sup>(379)</sup> Since the introduction of Troponin into clinical practice, several generations of commercial cardiac Troponin assays have been validated in analytical and clinical trials. In 2011, highly sensitive Troponin T (ROCHE) was introduced which is the 5<sup>th</sup> generation of the Troponin tests. This new more sensitive version of the assay - with a unit of measurement of nanogram per litre (ng/l) - has replaced the previous assays with unit of measurement of microgram per litre ( $\mu\text{g/l}$ ) in most cardiac centres in the UK. Development of newer high-sensitivity assays seems to have improved the value of cardiac troponin as both a diagnostic and a risk indicator.

At The Heart Hospital, high sensitive Troponin level was measured in  $\mu\text{g/l}$  with the 99<sup>th</sup> percentile upper reference limit of 0.014. In the other two recruiting centres (St Thomas' Hospital and The Essex Cardiothoracic Centre), the new hsTnT level was measured in  $\text{ng/l}$  with the 99<sup>th</sup> percentile upper reference limit of 13 and 14  $\text{ng/l}$  respectively. This new high sensitive Elecsys Troponin T assay, met the sensitivity criteria required by ESC/ACC, in achieving less than 10% coefficient of variation (CV) at the 99 percentile upper reference limit of the reference population .

As explained above, the unit of measurement at St Thomas' Hospital and The Essex Cardiothoracic Centre was nanogram per litre whereas at The Heart Hospital, Troponin was measured in microgram per litre. For the purpose of analysis and unifying the units of measurements, Troponin results at the Heart Hospital were multiplied by 1000 in order to convert them to nanograms per litre. This conversion equalised the unit of measurement between the three recruiting centres.

CK-MB mass assay in all of the three recruitment centres was measured using Elecsys 2010 CK-MB STAT assay (Roche Diagnostics). Upper reference limit and measurement units however were different at the laboratories of St Thomas' Hospital, The Heart Hospital and The Essex Cardiothoracic Centre. The measurement unit of CK-MB at St Thomas' Hospital was in microgram per litre ( $\mu\text{g/l}$ ). The reference range was different for men and women: 0.1-4.94  $\mu\text{g/l}$  for males and 0.1- 2.88  $\mu\text{g/l}$  for females. At The Heart Hospital the unit of measurement was  $\mu\text{g/l}$ , the reference range was 0-2.9 for both male and female. At The Essex Cardiothoracic Centre, the measurement unit was international unit/litre (IU/l) with a range of 0-25 for both male and female.

CK at St Thomas' Hospital was measured in IU/l and reference range was 0-229 in males and 0-159 in females. At The Heart Hospital, the unit of measurement for CK was IU/l with reference range of 26-140 for male and 38-104 for female. Basildon centre used IU with reference range of 40-320 for male and 25-200 for female. Pre-PCI (zero hour) blood samples were collected prior to applying the BP cuff inflation, as CK results could have been affected due to probable muscle injury post BP cuff inflation.

## **Secondary endpoint- Myocardial oedema and Necrosis**

In the ERIC-PCI trial we investigated whether RIPC in patients undergoing complex PCI will reduce PCI-related myocardial injury as evidenced by a reduction in the incidence and extent of myocardial oedema and necrosis in cardiac MRI. We hypothesised that Troponin elevation post PCI would represent myocardial injury evidenced by late gadolinium enhancement and myocardial oedema. We hypothesised that presence of LGE in the myocardium correlates with the Troponin release and the myocardial oedema detected by T2 mapping/STIR, represents acute myocardial injury during PCI. We used the CMR technique to determine whether our cardio-protective interventions reduced myocardial infarct size and myocardial oedema in the clinical settings of complex PCI.



## CMR Protocol

All CMR scans were performed on a 1.5-T Siemens scanner 1-7 days before and up to 7 days after PCI. The protocol consisted of:

1. Scout imaging.
2. Axial, coronal and sagittal views.
3. Steady-state free precession cine images were acquired in 2 long-axis and short-axis views from base to apex. The acquisition of short-axis views began 1 cm below the level of the mitral valve insertion plane and continued in 1-cm increments through the left ventricle.
4. A full stack of matched short-axis slices, 10 mm apart, was acquired for T2 maps to cover the left ventricle from base to apex.
5. A gadolinium-based contrast agent (Dotarem, Guerbet, France) was then administered intravenously at a dose of 0.1 mmol/kg body weight at 3 mls/second.
6. Early gadolinium enhancement images were taken immediately after contrast injection to exclude the presence of LV thrombus. Then contrast-enhanced images were acquired after a 10-minute delay with the use of an inversion-recovery segmented gradient echo sequence (FLASH). Contrast-enhanced images were acquired in identical long- and short-axis planes to the cine images.
6. A full stack of matched short-axis slices, 10 mm apart, was acquired for LGE to cover the left ventricle from base to apex.

Within 2-7 days after successful PCI, the second CMR was performed to assess for new late gadolinium enhancement and oedema. All patients were scanned by the researcher, apart from one occasion that due to commitment in another research centre, the CMR was performed by a radiographer.

## **Secondary Endpoint- Contrast-Induced Acute Kidney Injury**

In the ERIC-PCI trial we investigated whether RIPC in patients undergoing complex PCI would lead to a reduction in the incidence and severity of contrast-induced acute kidney injury. Currently, the diagnosis of CI-AKI is dependent on determining changes in serum creatinine level. The serum creatinine values however do not rise immediately after the contrast insult. The emergence of novel biomarkers offers an opportunity to diagnose CI-AKI at an earlier stage, which can differentiate between structural and functional AKI, and predict the outcome of established AKI.<sup>(380)</sup>

The ERIC-PCI trial was designed to assess the effect of RIPC in reducing CI-AKI by measuring blood creatinine and NGAL (Neutrophil Gelatinase associated Lipocalin). The incidence of CI-AKI was defined as a post-PCI increase in serum creatinine  $\geq 25\%$  or  $\geq 44 \mu\text{mol/L}$  from the baseline.  
(381, 382)

In all three centres, the creatinine was measured using the Roche Jaffe method, with the unit of measurement of  $\mu\text{g/l}$ . The reference range varied between the three centres. At St Thomas' Hospital, the reference range was 59-104  $\mu\text{g/l}$  for male and 45-84  $\mu\text{g/l}$  for female. At The Essex Cardiothoracic Centre, the reference range for creatinine was 46-80  $\mu\text{g/l}$  for both male and female.

NGAL samples were collected in Ethylene diamine tetra acetic Acid (EDTA) bottles before PCI and at 6, 12 and 24 hours post PCI. EDTA blood samples for NGAL were spun and centrifuged; plasma was aliquoted, and stored in a  $-20^{\circ}\text{C}$  freezer in the laboratory of the recruiting hospitals. According to the protocol, it was decided to courier NGAL blood samples to a core laboratory at UCLH for analysis.

## **Secondary endpoint- Chest Pain and ECG changes**

In the ERIC-PCI trial we investigated whether RIPC in patients undergoing complex PCI results in less peri-PCI chest pain and fewer procedure related ECG changes suggestive of myocardial ischaemia.

## **Secondary endpoint- MACE at 30 days**

In the ERIC-PCI trial we investigated whether RIPC in patients undergoing complex PCI reduces the rates of coronary revascularisation, re-infarction, and cardiovascular death at 30 days. The follow-up data were obtained from a clinical interview over telephone by the main researcher.

## **Coronary Angiography Report**

The angiographic findings and PCI procedure details were reported by two operating interventionalists - who were blinded to the RIPC treatment allocation- using local standard reporting modules. Presenting complaint, past history, arterial access site, PCI details i.e lesion(s) treated, balloons/stents used and any possible complications were documented in the report. Cardiac clinical physiologists also separately documented details of the procedure.

## CMR Analysis

CMR images were analysed by an experienced examiner blinded to the treatment allocation, previous revascularisation and recent angiographic findings. The second reporter was the main researcher who was not blinded to the treatment allocation, previous revascularisation and recent angiographic findings. The report was performed using commercially available software. Cine images were used to detect hypokinetic, akinetic or normal wall contractility. LV function was analysed following planimetry of all the short-axis cine images to determine left ventricular end-diastolic volume index, left ventricular end-systolic volume index and ejection fraction (EF) in %. Papillary muscles and pericardial fat were excluded from calculations.

Presence or extent of myocardial fibrosis (compared with pre-PCI CMR) was assessed by looking for areas of LGE on each of the short-axis and long axis views. MI size equals the total area of LGE expressed as a percentage of the total area of LV myocardium. In the ERIC-PCI study, an expert consultant identified the late gadolinium enhancement visually without use of automated quantification techniques.

T2-mapping CMR was used to measure the extent of myocardial oedema, as this sequence has been reported to be less susceptible to the imaging artefacts associated with traditional black-blood T2-weighted CMR sequences.<sup>(82, 331)</sup> MI size equalled the total area LGE expressed as a percentage of the total area of LV myocardium. Combination of late gadolinium enhancement and T2-weighted images were used as a clinically reliable method to differentiate acute from chronic MI. Although late gadolinium enhancement is a powerful marker of non-viability and detects infarction at any disease stage, transmural high T2 signal accurately identifies the area of the acute event.

## **Reporting of Adverse Events**

A Serious Adverse Event is any adverse event/experience occurring at any study that results in any of the following outcomes: death, life-threatening (subject at immediate risk of death), in-patient hospitalisation or prolongation of existing hospitalisation, persistent or significant disability or incapacity, OR important medical events that may not result in death, life-threatening or hospitalisation but may be considered a serious adverse event/experience when based upon appropriate medical judgment- they may jeopardise the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. According to the study protocol, any possible serious adverse events or reactions would need to be reported to the principal investigator and the R&D and ethics committee.

## **Confidentiality in Data Handling and Record Keeping**

Identifiable details which were collected from subjects in this study included: name, date of birth, hospital number, address and telephone number. The information was used to enable contact with participants. The study protocol, documentation, data and all other information generated were held in strict confidence. Confidential data was stored in a password-secured computer database. The Investigator was the custodian of the data. CD copies of CMR were anonymised and identified by case numbers. MRI scans were analysed at the CMR centre at The Heart Hospital. Coronary intervention procedures were reported immediately post procedure at each centre.

## Statistical Analysis

Data analysis was performed using the IBM SPSS statistics software package, version 21. In order to check the normal distribution between the two groups, histograms with normal plots were used. The differences between the groups for continuous variables were analysed by Analysis Of Variance (ANOVA). Chi-square test was used for categorical variables. The area under the curve (AUC) analysis was performed using the trapezoid rule and the significance interpreted using P value and the 95% confidence interval.

## Sample Size Determination

### a) Proposed sample size for PCI-related myocardial injury

As per the protocol of this study, PCI-related myocardial injury was planned to be detected and quantified by the measurement of hsTrop-T and use of cardiac MRI. The initial sample size calculation was performed based on the incidence of myocardial injury. In the Hoole et al study<sup>(15)</sup>, it was demonstrated that the median levels of serum Trop I at 24 hours post-PCI was reduced from 0.16 to 0.06 ng/ml (a 62.5% reduction) in patients treated with RIPC. In a pilot study of 42 unselected patients (stable, unstable and NSTEMI) undertaken at St Thomas' Hospital, the 24 hour- hsTropT level was  $1.86 \pm 2.24$  µg/L. In order to achieve a 62.5% reduction in hsTropT in patients undergoing PCI with 80% power and 0.05% significance level, 59 patients required per group.

According to the RIPC study by Hoole et al<sup>(15)</sup>, the proportion of patients with detectable levels of serum Trop I at 24 hours post-PCI was reduced from 71% in control to 52% with RIPC. To

achieve a similar reduction in our study with 80% power and 0.05% significance level, 102 patients per group were required. Therefore based on the above data, we aimed to recruit 100 patients per group.

### **b) Proposed Sample Size for contrast induced –acute kidney injury**

CI-AKI in this study was planned to be detected and quantified by the measurement of serum creatinine and NGAL. The sample size calculation was performed based on the incidence of CI-AKI or the extent of CI-AKI.

In terms of the extent of CI-AKI, a recent study demonstrated that RIPC can reduce the area-under-curve serum creatinine in patients undergoing elective surgery for repair of an abdominal aortic aneurysm.<sup>(375)</sup> In this study, it was reported that RIPC reduced the 7 day AUC serum creatinine from 1034±718 to 696±188 µmol/L (33% relative reduction). In order to achieve similar reduction in serum creatinine, in patients undergoing PCI in our study, with 80% power and 0.05% significance level, 71 patients were required per group.

In terms of incidence of CI-AKI, a recent study had shown that atorvastatin treatment prior to PCI reduced the incidence of contrast induced nephropathy (CIN) from 13.2 to 5%.<sup>(382)</sup> Therefore, to investigate whether RIPC had a similar effect on the incidence of AKI post-PCI, a total sample size of 302 patients (152 in each group) were required to provide 80% power to detect the difference with an alpha level of 0.05. However, in patients who were eligible to receive CIN prophylaxis (i.e. those with eGFR<60 mm/min/kg) the incidence of CIN post-PCI was increased and had been estimated at 20%. Therefore, to reduce the incidence of CI-AKI in this patient group by 50%, we required 108 patients per group with 80% power and 0.05% significance level. Thus, based on the above data we aimed to recruit 108 patients per group.

# **CHAPTER 4**

## **RESULTS**

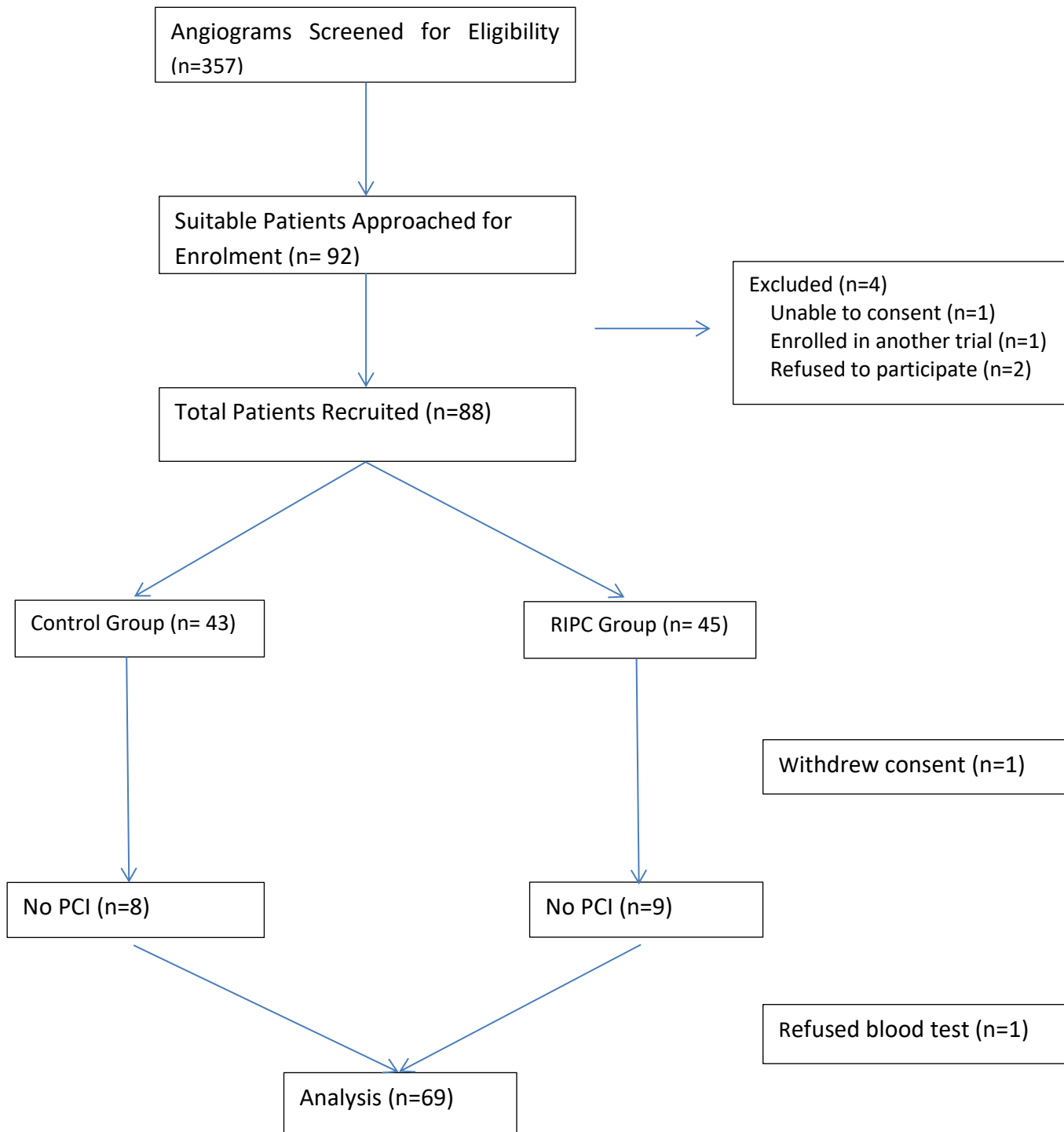


## Overview

Between April 2011 and August 2013, a total of 357 consecutive patients awaiting elective PCI at 3 cardiac centres (St Thomas' Hospital, The Heart Hospital and Basildon Cardiothoracic Centre) were screened for suitability for enrolment in the ERIC-PCI randomised-controlled trial. Patients with severely calcified coronary arteries requiring rotational atherectomy, with chronic occlusion of coronary arteries (CTO), with SYNTAX score between 23 and 35, or with stenosis of bypass vein grafts were considered eligible. Most of the eligible patients belonged to St Thomas' Hospital. Suitable patients were shared equally amongst multiple research studies running simultaneously at this centre. Out of 357 screened patients, 92 patients were approached for participation in the ERIC-PCI study. A total of 88 patients were successfully recruited in the study, 43 patients were allocated in the control group and 45 in the RIPC group.

The flow chart below shows the process of screening and recruitment.

## ERIC-PCI Study Flow Chart



## Patients' Profile and Baseline Characteristics

Patients' profile and baseline characteristics were well balanced and evenly distributed in both control and RIPC groups. No statistically significant differences were noted between the two groups with respect to the majority of the baseline parameters such as age and gender. Patients had ongoing stable symptoms despite anti-anginal medications. Patients' characteristics are explained in details below and summarised in table 4-1.

The difference in the medical background was not significant between the two groups in terms of smoking, hypertension, diabetes, dyslipidaemia, renal function, previous PCI, CABG or cerebrovascular disease.

There was a difference in the left ventricular systolic function between the two groups. 14% of patients in the control group and 37 % of patients in the RIPC group had good LV systolic function. In 11.6 % of patients in the control group and 4.4% of patients in the RIPC group, the LV function was poor. The LV function was unknown in 69.8% of patients in the control group and 48.9% of patients in the RIPC group. (P =0.03)

All patients were stable and none of them had suffered from angina or raised Troponin level prior to the elective PCI.

**Table 4.1- Baseline Characteristics of Patients**

Demographics	Control	RIPC	P value
Age (years)	66.3±12.1	67.6±12.5	0.37
Male	40 (93.0%)	37 (82.2%)	0.12
Female	3 (7%)	8 (17.8%)	
BMI(Kg/m <sup>2</sup> )	29.9 ± 6.9	28.9±5.5	0.54
Smoking History	21 (51.2%)	20 (48.8%)	0.60
Hypertension	35 (81.4 %)	38 (84.4%)	0.78
Dyslipidaemia	43 (100%)	42 (93.3%)	0.24
Diabetes	13 (30.2%)	14 (31.1%)	1.0
LV function			0.03
Good	6 (14%)	17 (37.8%)	
Fair	2 (4.7%)	4 (8.9 %)	
Poor	5 (11.6%)	2 (4.4 %)	
Unknown	30 (69.8%)	22 (48.9%)	
Baseline creatinine (µmol/l)	89.36± 19.0	84.76± 26.5	0.49
Prior PCI	12 (27.9%)	10 (22.2%)	0.62
Prior CABG	14 (32.6%)	13 (28.9%)	0.80
Prior CVA	3 (7%)	2 (4.4%)	0.67

## Pre-PCI Medications Profile

Medications commonly prescribed in cardiovascular disease, have an influence on RIPC, either by mimicking the protective effect of RIPC and potentiating the preconditioning effect or by abolishing the effect of RIPC. Anti-platelets, Beta-blockers, Statins, Nitrates, Nicorandil and ACE inhibitors may precondition the myocardium. <sup>(314)</sup> Antidiabetic medications such as glibenclamide can disrupt cardioprotection through the inhibition of ATP-dependent potassium channels, therefore blocking cardioprotection. <sup>(323, 324)</sup> (Table 4.2)

In the ERIC-PCI trial, there was no significant difference between the two groups in terms of medicines taken. We advised patients to withhold nicorandil and glibenclamide 48 hours prior to elective PCI. Anti-platelets, Beta-blockers, Statins, Nitrates and ACE inhibitors were continued.

**Table 4.2-Pre-PCI Medications Profile**

<b>Demographics</b>	<b>Control</b>	<b>RIPC</b>	<b>P value</b>
Aspirin	42 (97.7%)	38 (88.4%)	0.10
Clopidogrel	18 (41.9%)	16 (37.2%)	0.80
Prasugrel	0 (0%)	1(2.3%)	1.00
Warfarin	3 (7%)	6 (14%)	0.48
Statin	40 (93%)	37(86%)	0.48
Beta-blocker	30 (69.8%)	32 (74.4%)	0.81
Oral Nitrate	18 (41.9%)	17 (40.5%)	1.00
Nicorandil	6 (14%)	9(20.9%)	0.57
Ranolazine	2 (4.7%)	1 (2.3%)	1.00
ACEI/ARB	33 (76.7%)	33 (76.7%)	1.00
Metformin	9 (20.9%)	8 (18.6%)	1.00
Insulin	2 (4.7%)	1 (2.3%)	1.00

## Anti-platelets

Inhibition of platelet activation with Aspirin is fundamental in coronary interventions as it is associated with decreased incidence and significance of acute coronary thrombosis. <sup>(383)</sup>

Combination of aspirin and P2Y12 receptor inhibitors, called 'dual anti-platelet' therapy, is the gold standard management after placement of coronary stents. <sup>(123-127)</sup> Observational registries have confirmed that Clopidogrel decreases PCI related ischaemic events. <sup>(384, 385)</sup> Therefore, patients are required to be on dual antiplatelet therapy; Aspirin combined with Clopidogrel, Prasugrel or Ticagrelor at the time and after PCI.

In the ERIC-PCI trial, 42 patients in the control group (97.7%) and 38 patients in the RIPC group (88.4%) were on long-term treatment with Aspirin. The two control and RIPC groups were not statistically different in this characteristic,  $P = 0.10$ .

Eighteen patients in the control group (41.9%) and 16 patients in the RIPC group (37.2%) were on long-term treatment with Clopidogrel prior to PCI,  $P = 0.80$ . Only one patient in the RIPC group was on treatment with prasugrel and no patient was treated with ticagrelor. Patients, who were not on the maintenance dose of anti-platelets, received a loading dose of Aspirin 300 mg and/ or Clopidogrel 600 mg on the ward prior to PCI.

## Statins

Administration of Statins prior to PCI is effective in reducing myocardial damage during coronary interventions. <sup>(386,387)</sup> Statins are effective through different mechanisms other than lipid lowering. The anti-inflammatory role of statins is more prominent and may influence PCI related events, reduce PCI-related myocardial injury and improve outcomes. <sup>(386)</sup>

In the ERIC-PCI trial, 40 patients in the control group (93%) and 37 patients in the RIPC group (86%) were on treatment with statin. The two control and RIPC groups were not statistically different in this characteristic, P = 0.48.

## **Beta -blockers**

Benefit from beta- blockers in reduction of myocardial necrosis has been suggested in both experimental and clinical studies. The potential of beta-Blockers to limit myocardial necrosis was initially proposed in 1972 by Sommers et al. <sup>(388)</sup> followed by Reimer et al. in 1973. <sup>(389)</sup> After a few years of conflicting results and debate about the efficacy of  $\beta$ -Blockers, the effective role of intra-coronary beta-blockers in protecting the heart from ischaemia/reperfusion injury is now confirmed and accepted. <sup>(390-394)</sup> Regarding the benefit of long-term treatment with oral beta-blockers, the results can be conflicting. <sup>(91)</sup>

In the ERIC-PCI trial, 30 patients in the control group (69.8%) and 32 patients in the RIPC group (74.4%) were on long-term treatment with beta-blockers. The two control and RIPC groups were not statistically different in this characteristic, P = 0.81.

## **Nitrates**

Nitrates are known to have preconditioning effect. Gori <sup>(393)</sup> Nitrates can protect myocardium from ischaemia and reperfusion through a mechanism similar to preconditioning. In the ERIC-PCI study, 18 patients in the control group (41.9%) and 17 patients in the RIPC group (40.5%) were on long-term treatment with oral nitrate. The two control and RIPC groups were not statistically different in this characteristic, P = 1.0.



## Nicorandil

As per Yellon's laboratory finding, Nicorandil can mimic the protection of ischaemic preconditioning.<sup>(156)</sup> This effect is similar to the effect of other potassium channel openers that by mimicking preconditioning can protect the myocardium for ischaemic/reperfusion injury or distal embolisation. Nicorandil dilates coronary microcirculation, induces ischaemic preconditioning, is antiarrhythmic and reduces reperfusion injury via the adenosine triphosphate (ATP)-sensitive K<sup>+</sup> channel.<sup>(157, 158)</sup> In a large randomised-controlled trial in STEMI patients, intravenous Nicorandil resulted in significant improvement in TIMI flow, ST segment resolution and final infarct size as measured by CK post PCI.<sup>(394)</sup>

Therefore, we advised the ERIC-PCI trial patients to withhold Nicorandil at least 48 hours prior to the day of PCI procedure.

## Glibenclamide

Sulphonylurea antidiabetic drugs such as Glibenclamide can disrupt cardioprotection through the inhibition of ATP-dependent potassium channels; therefore, cardioprotection can be blocked and abolished.<sup>(323, 324)</sup> According to the ERIC-PCI protocol, patients should have ideally stopped glibenclamide 24-48 hours prior to PCI. None of the patients was on long-term treatment with glibenclamide though. According to the laboratory findings, Glimepiride is able to potentiate and facilitate the ischaemic preconditioning effect.<sup>(395)</sup> Diabetic hearts are resistant to the IPC effect but Glimepiride has been shown to overcome this resistance.

In the ERIC-PCI study, no patient was on long-term treatment with Glimepiride.

## **Metformin**

Metformin is known to confer cardioprotection in both diabetic and non-diabetic hearts. The cardioprotective mechanism of Metformin is through inhibition of mitochondrial permeability transition pore opening.<sup>(396)</sup>

In the ERIC-PCI trial, 9 patients in the control group (20.9%) and 8 patients in the RIPC group (18.6%) were on long-term treatment with Metformin. The two control and RIPC groups were not statistically different in this characteristic,  $P = 1.0$ .

## **Angiotensin-Converting Enzyme Inhibitors (ACE-Inhibitors)**

Yellon's laboratory in 1997 showed that ACE-Inhibitors in combination with a subthreshold preconditioning stimulus, potentiated the ischaemic preconditioning effect through Bradykinin B2 receptor.<sup>(397)</sup> Pre-treatment with ACEIs however did not show significant reduction in infarct size.

In the ERIC-PCI trial, 33 patients in the control group (76.7%) and 33 patients in the RIPC group (76.7%) were on long term treatment with ACE-Is. The two control and RIPC groups were not statistically different in this characteristic,  $P = 1.0$ .

## Coronary Intervention Variables

There were no major differences between the PCI procedures of the two treatment groups

(Table 4.3)

**Table 4.3- Peri-PCI variables**

Variable	Control	RIPC	P value
Target vessel			
RCA	39.5%	33.3%	0.67
LAD	23.3%	31.1%	
Vein graft	14%	13.3%	
LMS	11.6%	4.4%	
Cx	7%	11.1%	
Multi-vessel	2.3%	4.4%	
Diagonal	2.3%	2.4 %	
Intervention			
Rotablation PCI	34.9%	35.6%	0.92
CTO PCI	20.9%	17.8%	
Attempted CTO PCI	11.6%	17.8%	
PCI to Vein graft	9.3%	6.7%	
Laser PCI	4.7%	2.2%	
PCI in SYNTAX >23	11.6%	14.5%	
Abandoned PCI	7%	4.7%	
ACC/AHA Lesion Type			
A	40 %	41.5%	0.48
B	39.4%	40.9%	
C	20.6%	17.6%	

Rentrop Grade			
0	50 %	42.9 %	
1	17.6%	29.9%	0.59
2	26.5 %	24.3 %	
3	5.9%	2.9%	
TIMI grade score pre-PCI	1.3 ±1.9	1.7 ±1.1	0.18
TIMI grade score post-PCI	2.8 ±0.7	2.7 ±0.7	0.79
Wires (n)	2 ±0.7	1.9 ±0.8	0.67
Stents (n)	2.0 ±1.0	1.9 ±1.3	0.66
Pre-dilation time (seconds)	49.7±35.9	38.5±26.6	0.17
Post-dilation time (seconds)	18.2±21.4	19.7±25.2	1.0
Procedure time (min)	54.2±27.1	56.1±34.1	0.98
Screen time (min)	19.1 ± 13.7	19.2±15.3	0.98
Radiation dose cGycm <sup>2</sup>	6516.8± 4410	8951±3381	0.50
Burr Duration (seconds)	71.3 ±18.8	74.8 ±13.1	0.6
Burr Size (mm)	1.5 ±0.16	1.6 ±0.21	0.51

**Table 4.4- Peri-PCI Immediate Complications**

<b>Complication</b>	<b>Control</b>	<b>RIC</b>	<b>P value</b>
Dissection	1 (2.8%)	1 (2.8%)	1.0
Jailed Side Branch	2 (5.6%) and	3 (8.3%)	1.0
Distal Embolisation	1 (2.8)	0 (0%)	
Brief Chest Pain	2(4.7%)	3 (6.7%)	0.76
Prolonged Chest pain (> 20 minutes)	0(0%)	0(0%)	1.0
ECG changes			
ST elevation>1 mm	1 (2.3%)	2 (4.4%)	0.80
ST Depression	0 (0%)	1 (2.2%)	
VT/VF	2 (4.7%)	1 (2.2%)	

## **Treated Vessels and PCI Intervention**

The most commonly treated vessel in the ERIC-PCI trial was the right coronary artery (36.4%), followed by the left anterior descending artery (27.3%), saphenous vein grafts (13.6%) and the circumflex artery (9.1%). The two control and RIPC groups were not statistically different in this characteristic, P =0.67.

Rotational atherectomy assisted PCI was the most frequent procedure performed in the ERIC-PCI trial (35.2%). Fifteen patients in the control group (34.9%) and 16 patients in the RIPC group (35.6%) were treated with rotational atherectomy. Laser assisted PCI was the least frequent interventional procedure performed in the ERIC-PCI trial. In total three patients had laser assisted PCI; two in the control group and one in the RIPC group. The two control and RIPC groups were not statistically different in this characteristic,  $P = 0.92$ .

## **Stent Type**

Drug eluting stents (DES) coated with an anti-proliferative drug (usually paclitaxel, everolimus, sirolimus, zotalolimus) inhibit the neointimal proliferation after PCI by sustained local delivery of anti-proliferative drugs which results in less in-stent restenosis.<sup>(398)</sup> The efficacy of DES in prevention of re-stenosis is superior to bare metal stents (BMS) without coatings.<sup>(399)</sup> In the ERIC-PCI trial, four patients in the control group (9.3%) and seven patients in the RIPC group (15.6%) were treated with bare metal stents. Thirty-one patients in the control group (72.1%) and twenty-one patients in the RIPC group (60%) had drug eluting stents. The two control and RIPC groups were not statistically different in this characteristic,  $P = 0.51$ .

## **Stent Parameters (Diameter and Length)**

Stent parameters predict adverse events following PCI. The length of the implanted stent correlates with the level of cardiac Troponin release.<sup>(400)</sup> Therefore, limiting the stent length by spot-stenting lesions rather than covering the entire vessel between lesions may reduce subsequent embolisation and side branch occlusion, which in turn reduces PCI related myocardial injury.

In the ERIC-PCI trial, the average stent length was  $49.4 \pm 24.9$  mm in the control group and  $42.6 \pm 24.4$  mm in the RIPC group. The two groups were not statistically different in this characteristic,  $P=0.36$ . The average stent diameter was  $3.3 \pm 0.3$  mm in the control group and  $3.3 \pm 0.5$  mm in the RIPC group. The two groups were not statistically different in this characteristic,  $P= 0.89$ .

## **Intra-Coronary Nitrate**

Intra-coronary nitrate is commonly used in the angiography laboratories for better estimation of the stent size and for management of coronary spasm. Nitrates which are also commonly used for angina treatment are well known for their preconditioning effect. Gori<sup>(393)</sup> In a human study by Gori et al. it was demonstrated that GTN protects the endothelium against post-ischaemic endothelial dysfunction in a mechanism that is mediated by oxygen free radical release and opening of mitochondrial permeability transition pores.<sup>(393)</sup> (See chapter 1 for further details)

In the ERIC-PCI trial, 59 patients (72.8%) received intra-coronary nitrate during PCI in the form of either Isosorbide di Nitrate or GTN. Of these, 28 patients were in the control group and 31 patients were in the RIPC group. There was no statistically significant difference between these two groups (68.3% vs 77.5%,  $P = 0.45$ )

## **Intracoronary Adenosine**

Adenosine has long been known to be a coronary vasodilator, and an effective agent in preventing no-reflow and ischaemia/reperfusion injury. <sup>(401)</sup> The cardioprotective role of intravenous and intra coronary Adenosine has been confirmed in a few animal and human studies. <sup>(402-404)</sup> Combined therapy with Adenosine and Nitroprusside or Nicorandil might provide better improvement in coronary flow compared to intracoronary adenosine alone in case of impaired flow during coronary interventions <sup>(405)</sup> Preconditioning using intracoronary administration of adenosine has been shown to decrease myocardial damage caused by elective PCI. <sup>(406)</sup>

In the ERIC-PCI trial, intracoronary Adenosine was administered in one patient only who was randomised to the RIPC group.

## **Balloon Inflation Post Stent Deployment**

Suboptimal deployment of stent can routinely be optimised by post-dilation. Incidence of incomplete stent deployment ranges from 20% to 30% of cases and adjunctive balloon dilation is necessary to improve the minimum stent area and the uniform volumetric stent expansion. Incomplete apposition and under expansion may contribute to thrombosis and restenosis.

PCI can induce coronary spasm. If intra-lesional spasm persists despite administration of intracoronary nitrate, a prolonged low-pressure inflation using a balloon matched to the reference segment is usually successful in alleviation of coronary spasm.



In the ERIC-PCI trial, in total 52 patients received balloon inflation post stent deployment (post-dilation). Of these, 25 were in the control group and 27 were in the RIPC group. There was no statistically significant difference between these two groups in this regard. (65.8% vs 71.1%, P = 0.80)

## **Glycoprotein IIb/IIIa**

Further inhibition of platelet activity, after treatment with aspirin and clopidogrel can be achievable by using glycoprotein (GP) IIb/IIIa receptor antagonists, which are very effective especially in interventional treatment of acute coronary syndromes. <sup>(407)</sup> In TOPSTAR trial, additional temporary peri- and post-procedural administration of the GP IIb/IIIa receptor antagonist tirofiban led to a reduced incidence of post-interventional troponin release in elective, non-acute PCI in patients pre-treated with aspirin and clopidogrel.<sup>(407)</sup>

In the ERIC-PCI trial, Glycoprotein IIb/IIIa was administered in one patient in the control group who underwent laser assisted PCI to SVG, complicated with distal vessel embolisation.

## **Contrast Media**

It is widely accepted that use of contrast media during coronary investigations and interventions is associated with nephropathy/acute kidney injury (CI-AKI). Experimental findings indicate that contrast media administration rapidly induces a renal vasoconstrictive response. This has been ascribed to a number of different mediators, such as the renin-angiotensin system, changes in the intracellular calcium concentration of smooth muscle cells, adenosine and endothelin. <sup>(352,353)</sup>

In the ERIC-PCI trial, the average amount of contrast media used in the control group was 229.8±93.9 mls and 196.7±102.4 mls in the RIPC group. The two control and RIPC groups were not statistically different in this characteristic, P = 0.23.

## **PCI- related immediate complications**

Dissection of the coronary artery occurred in 2 cases, 1 CTO case and 1 attempted CTO, one in the control group and one in the RIC group, p value=1.0

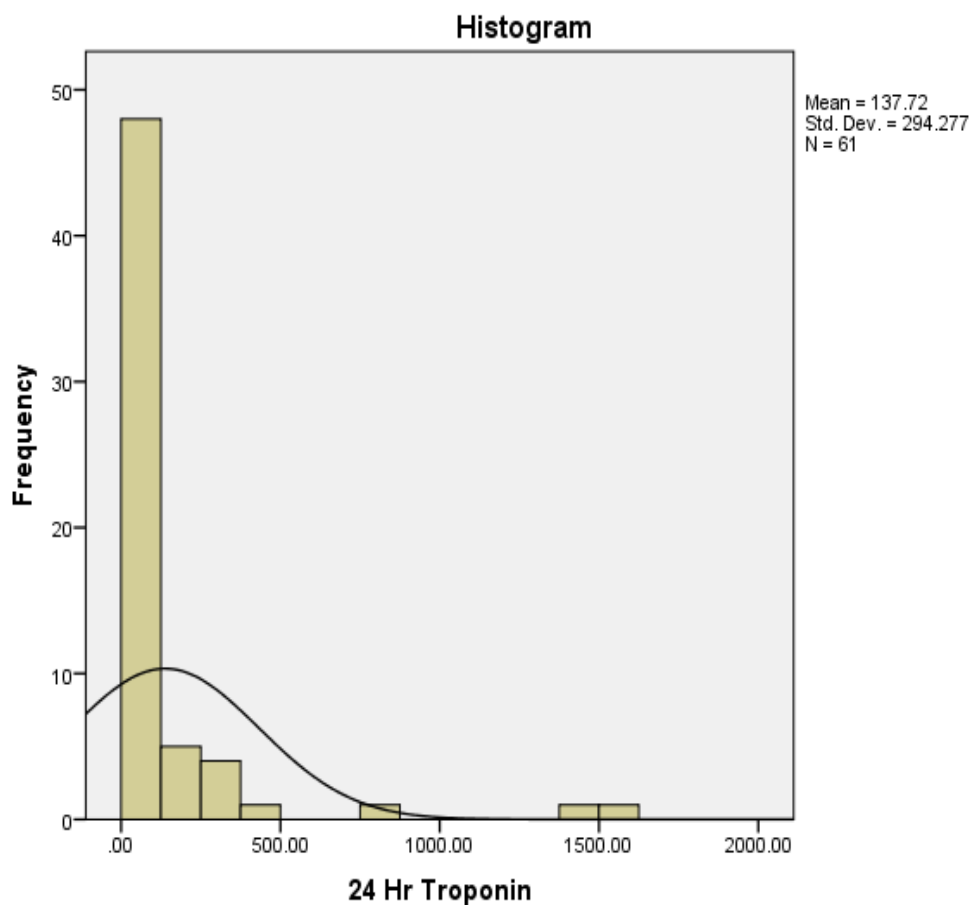
Jailed Side Branch occurred in 2 of the control group (5.6%) and 3 of the RIC group (8.3%), p value= 1

Distal embolisation developed in one patient in control group undergoing laser PCI. Patient received REOPRO.

## Primary End Point – Assessment of Troponin Level Post PCI

In the ERIC-PCI trial, high sensitive Troponin was measured at times zero (before the planned intervention), 6, 12 and 24 hours post PCI.

As seen in the histogram below, the total 24-hour Troponin results had a skewed distribution hence non-parametric tests were used for analysis.

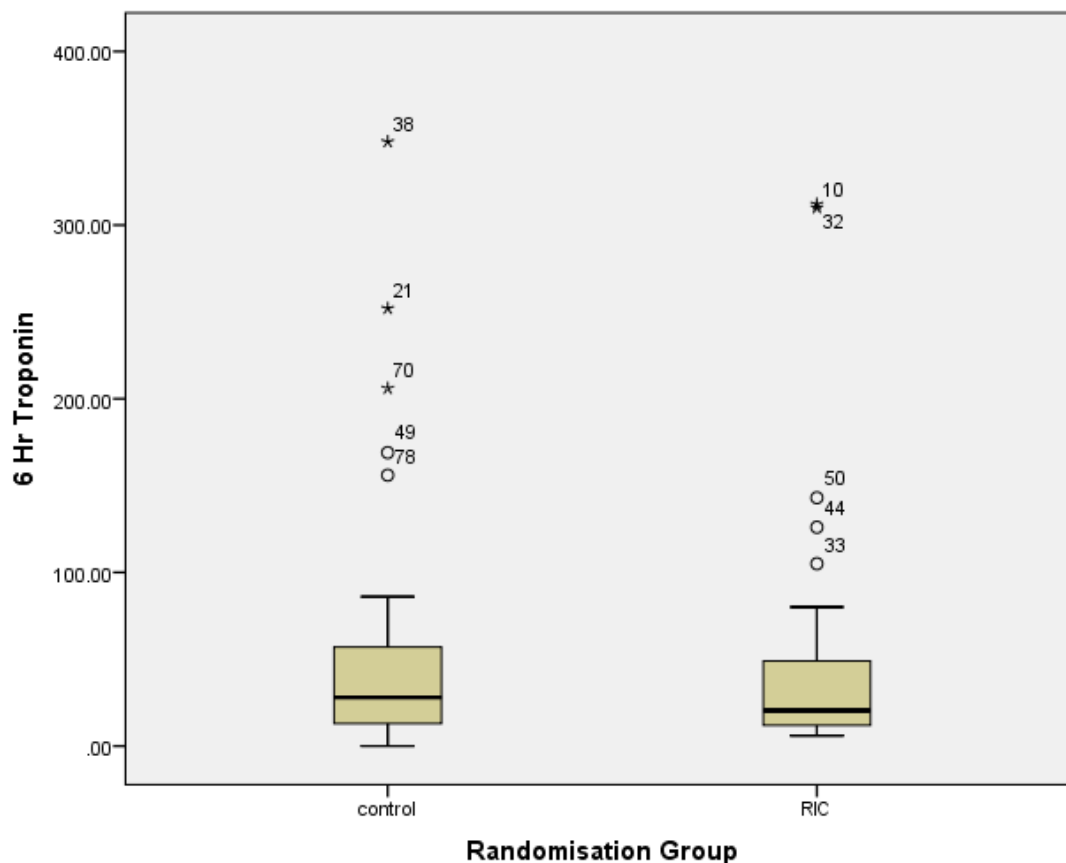


## No Significant Reduction in Troponin Release Post PCI

The median Troponin level at different time intervals as well as the distribution of Troponin across the two groups was assessed using the non-parametric Mann Whitney- U test. As seen in stem- leaf graphs below the Troponin release was lower in the RIPC group. This attenuation however was not statistically significant.

Each time set is analysed separately and shown as below:

### Six-hour Troponin results compared between the two groups



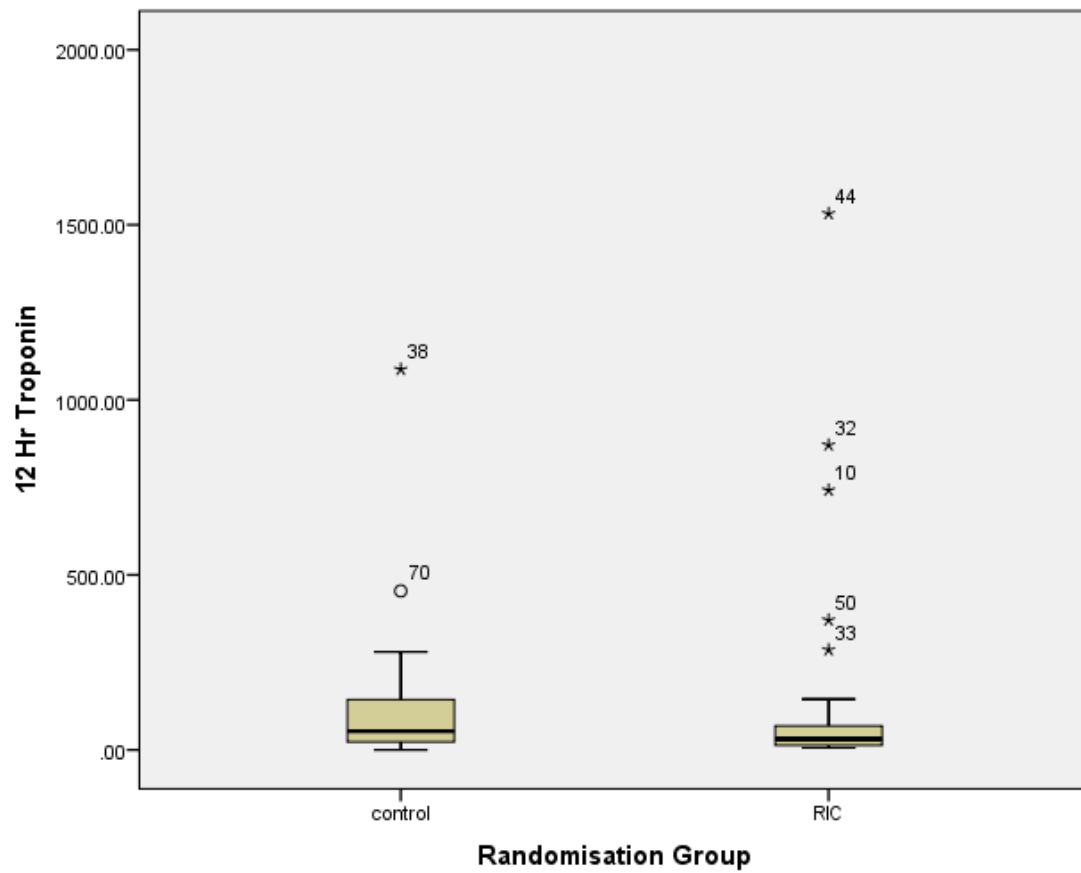
Outliers are

marked with a circle and the case number. Extremes are marked with a star and the case

number. There was no statistically significant difference between the two control and RIPC

groups, (P = 0.44). The significance level is 0.05.

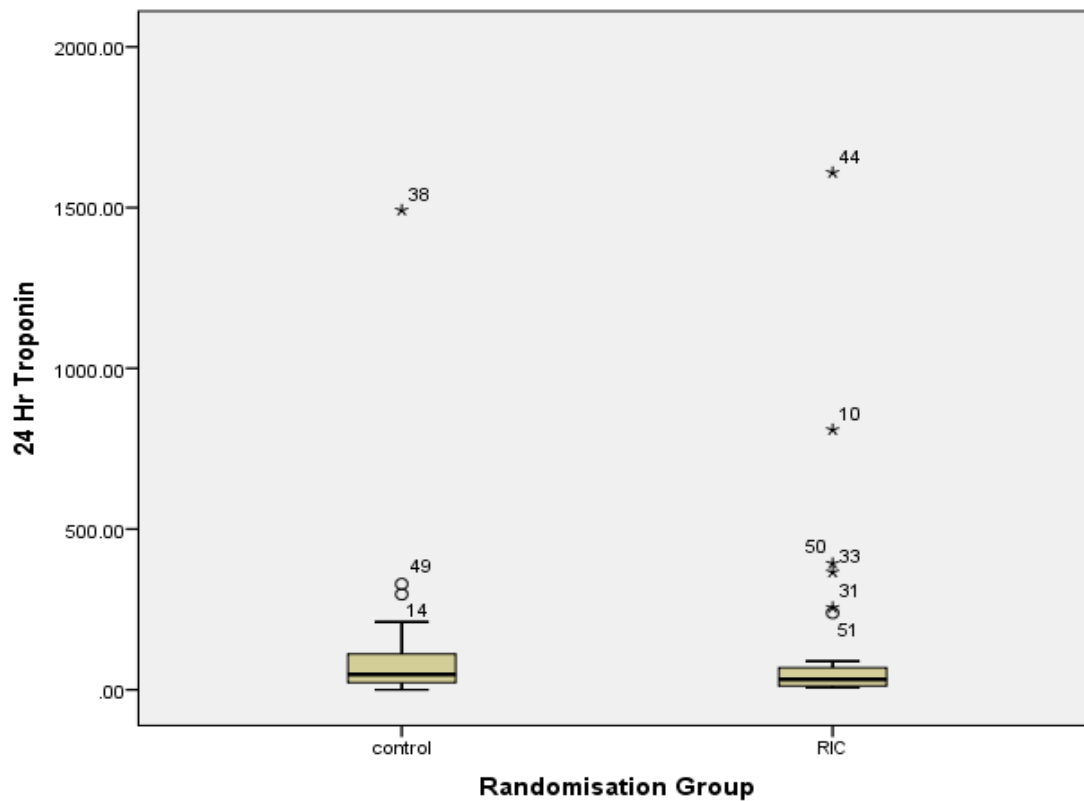
## Twelve-hour Troponin results compared between the two groups



Outliers are marked with a circle and the case number. Extremes are marked with a star and the case number.

There was no statistically significant difference between the two control and RIPC groups, (P = 0.31). The significance level is 0.05.

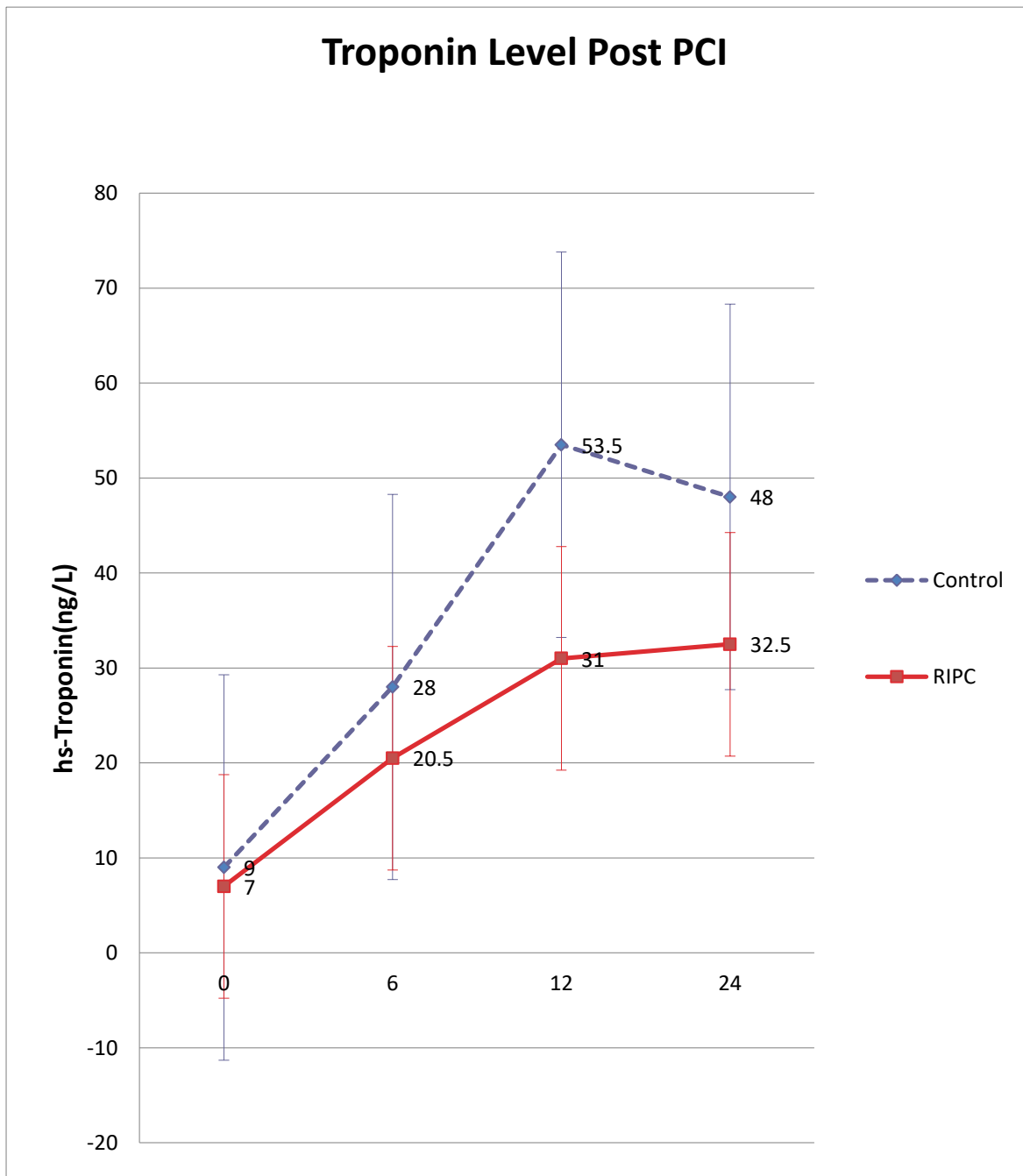
## Twenty-four hr Troponin results compared between the two groups



Outliers are marked with a circle and the case number. Extremes are marked with a star and the case number.

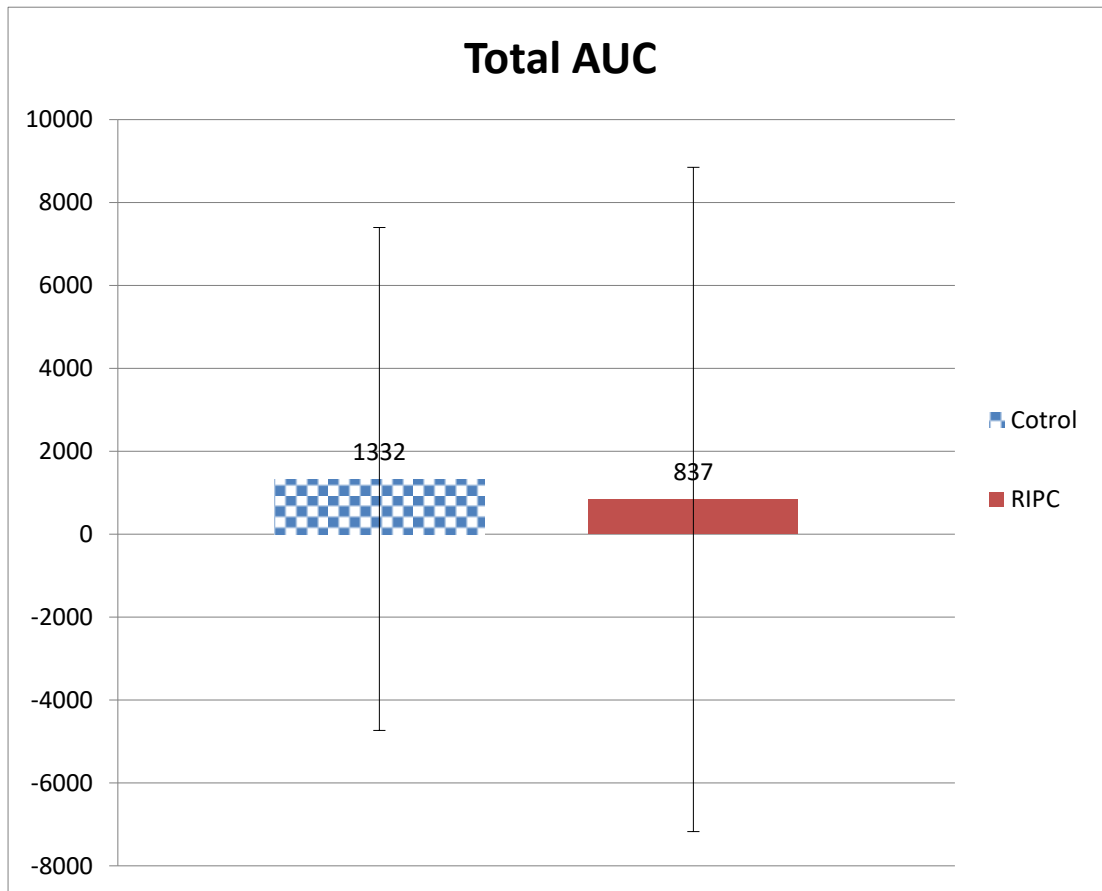
There was no statistically significant difference between the two control and RIPC groups, (P = 0.39). The significance level is 0.05.

**Graph 4.1 Troponin Level Over 24 Hours after PCI**



**For the P values please refer to the text on page 129**

**Graph 4.2 Area under the Curve (AUC) of 24-hour Troponin**



***P= 0.43***



The median high sensitive Troponin T level 24 hours post PCI, was 48 ng/l in the control group vs 32.5 ng/l in the RIPC group. This reduction in Troponin release -24 hours post PCI- was not statistically significant, P = 0.39.

Therefore, in the ERIC-PCI trial, RIPC failed to demonstrate a statistically significant attenuation of serum Troponin release. ‘

Troponin levels at 6 and 12 hours post PCI also had a similar pattern; although the enzyme release was less in the RIPC group, this attenuation was not statistically significant, P= 0.44 and 0.31 respectively for 6 and 12 hour results.

Furthermore, there was no significant reduction in the total area under the curve (AUC) in the RIPC group, P= 0.43. (Graph 4-2)

### **Primary Endpoint-Incidence of PCI-related myocardial injury**

In the ERIC-PCI trial, there was no statistically significant reduction in the incidence of troponin release in the preconditioned group.

PCI-related myocardial injury is defined as elevations of cTn >5 x 99th percentile upper reference limit (URL) occurring within 48 hours of the procedure in addition to: a) evidence of prolonged ischaemia (more than 20 min) as demonstrated by prolonged chest pain, or b) ischaemic ST changes or new pathological Q waves, or c) angiographic evidence of a flow limiting complication, such as a loss of patency of a side branch, persistent slow- flow or no-reflow, embolisation or d) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality. <sup>(3)</sup>

In the ERIC-PCI trial, measurement of Troponin level at 48 hours was not possible due to the logistic issues; therefore Troponin level were measured not later than 24 hours post PCI. Elevation of Troponin was calculated based on the baseline (zero-hour) Troponin and not the 99<sup>th</sup> percentile URL.

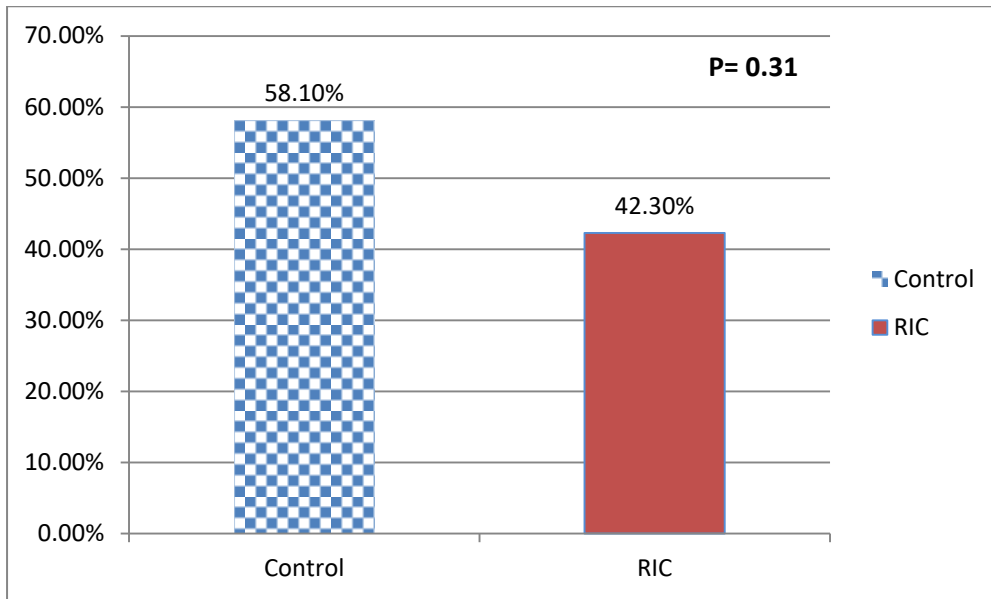
Significant elevation of Troponin level post PCI (> 5 x baseline) was observed in 26.1% of the whole recruited patients. This number is not dissimilar from the published literature that about one-third of all elective PCI procedures are associated with significant myocardial injury. In the control group, 46.9% and in the RIPC group, 26.7 % had significant rise of Troponin level. This observed attenuation in the PCI related Troponin release, was not statistically significant,  $p = 0.12$ .

Of these patients with significant elevation of Troponin level 24 hours post PCI, in one patient in the control group and in two patients in the RIPC group the side branch was lost during PCI (associated with ischaemic ST changes in ECG). One patient in the control group developed ventricular fibrillation (VF). Of these patients with significant elevation of Troponin level, no one suffered from chest pain more than 20 minutes.

The criteria below is required for diagnosis of type 4a MI: 1) elevations of cTn >5 x 99<sup>th</sup> percentile upper reference limit (URL) occurring within 48 hours of the procedure in addition to: 2) evidence of prolonged ischaemia (more than 20 min) as demonstrated by prolonged chest pain, or 3) ischaemic ST changes or new pathological Q waves, or 4) angiographic evidence of a flow limiting complication, such as a loss of patency of a side branch, persistent slow-flow or no-reflow, embolisation or 5) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality. Therefore in our trial, Type 4a MI was only observed in 3 patients, one in control and two in the RIC group.

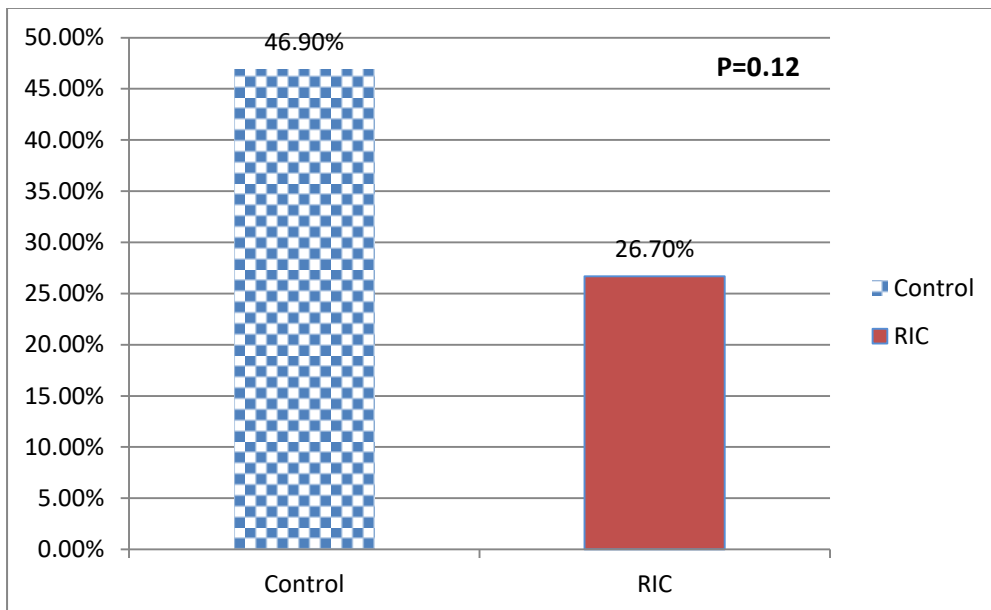
Based on the Second Global MI Task Force, leading to the Universal Definition of Myocardial Infarction Consensus Document in 2007, which suggested to designate increases more than three times the 99<sup>th</sup> percentile URL as PCI-related MI, elevation of Troponin level was observed in 69.3% of the whole recruited patients in the ERIC-PCI study; 72.1% in the control group and 66.7% in the RIC group. No statistical difference was observed, P = 0.31

**Graph 4.3 Incidence of MI based on 2<sup>nd</sup> definition of peri-PCI MI**



***P= 0.31***

**Graph 4.4 Incidence of MI based on 3<sup>rd</sup> definition of peri-PCI MI**



***P = 0.12***

## **Primary End Point – Assessment of CK-MB Level Post PCI**

Total analysis of CK-MB results of the whole recruited patients was not possible as different assays with different units of measurement were used by the laboratories of St Thomas' Hospital, The Heart Hospital and The Essex Cardiothoracic Centre.

CK-MB assay at St Thomas' Hospital measured the CK-MB mass, using the unit of measurement of microgram per litre ( $\mu\text{g/l}$ ). The reference range was different in men and women: 0.1-4.94  $\mu\text{g/l}$  for males and 0.1- 2.88  $\mu\text{g/l}$  for females.

The Heart Hospital also measured the CK-MB mass with the unit of measurement of  $\mu\text{g/l}$ . The reference range however did not differ between male and female; 0-2.9  $\mu\text{g/l}$  used for both gender.

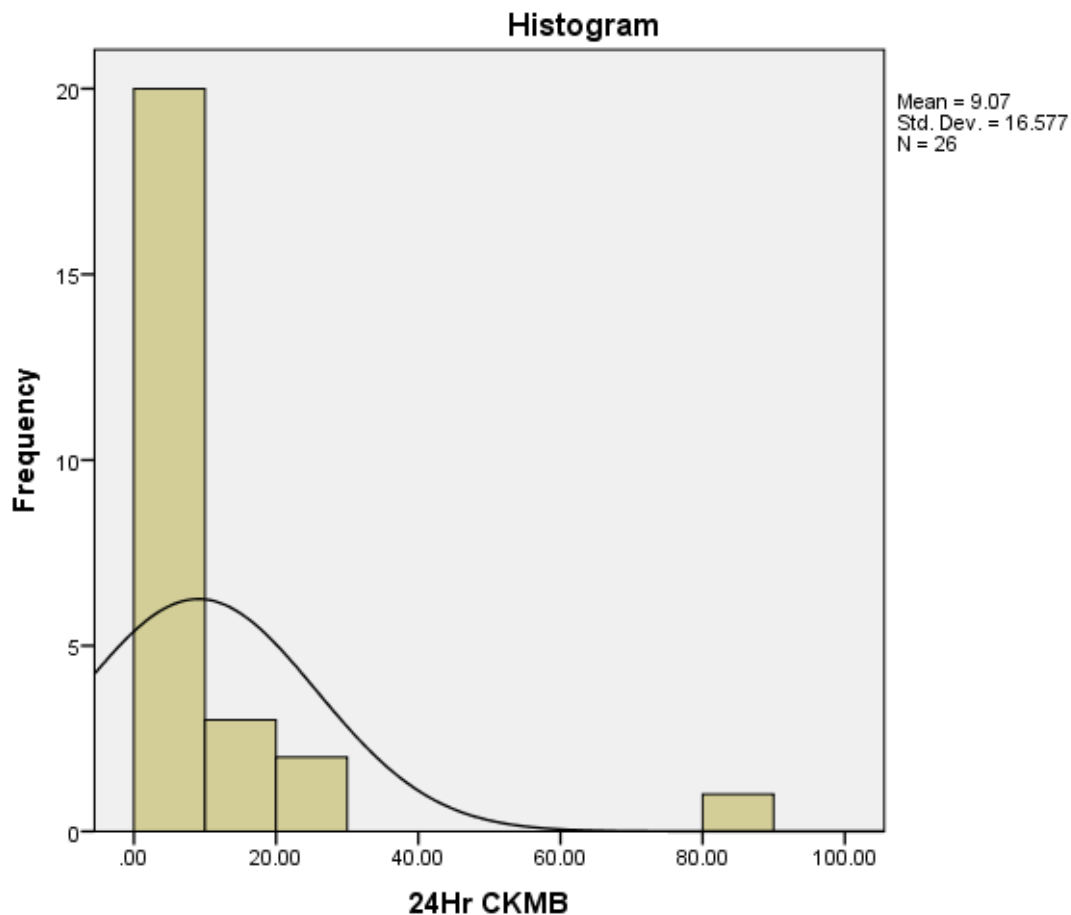
At The Essex Cardiothoracic Centre, the assay measured the activity of CK-MB using international unit/litre (IU/l) as the unit of measurement with a reference range of 0-25 for both male and female.

The mass and activity cannot be converted into each other. Hence, considering the above variations in the mass assay used at the three recruiting centres, analysis of CK-MB release was not possible.

However, as most of the ERIC-PCI trial patients were recruited at St Thomas' Hospital, CK-MB analysis at this centre has been performed as below.

## CK-MB analysis- St Thomas' Hospital

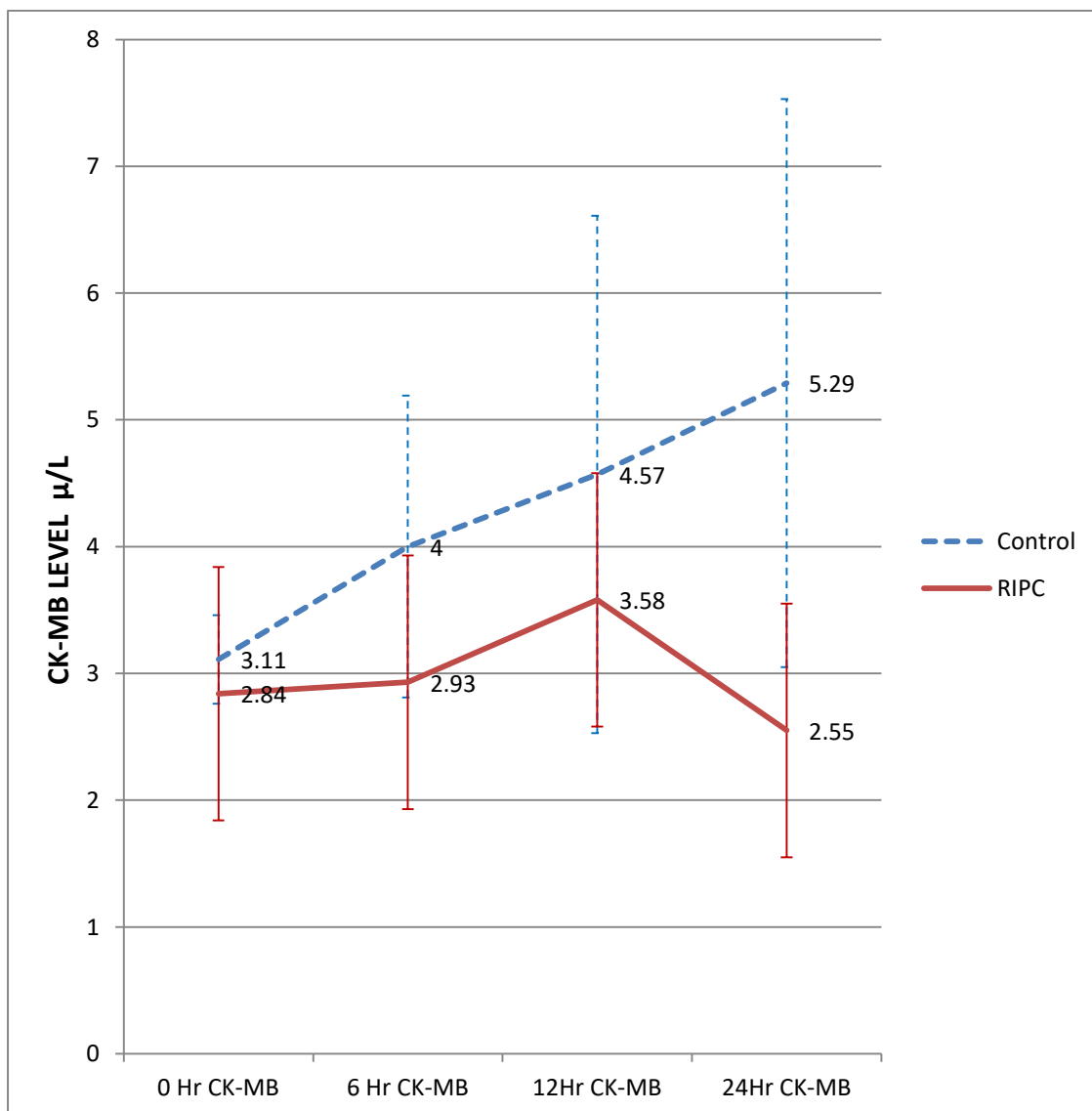
As seen in the histogram below, similar to the Troponin results, 24-hour CK-MB levels had a skewed distribution- - therefore non-parametric tests were used for analysis.



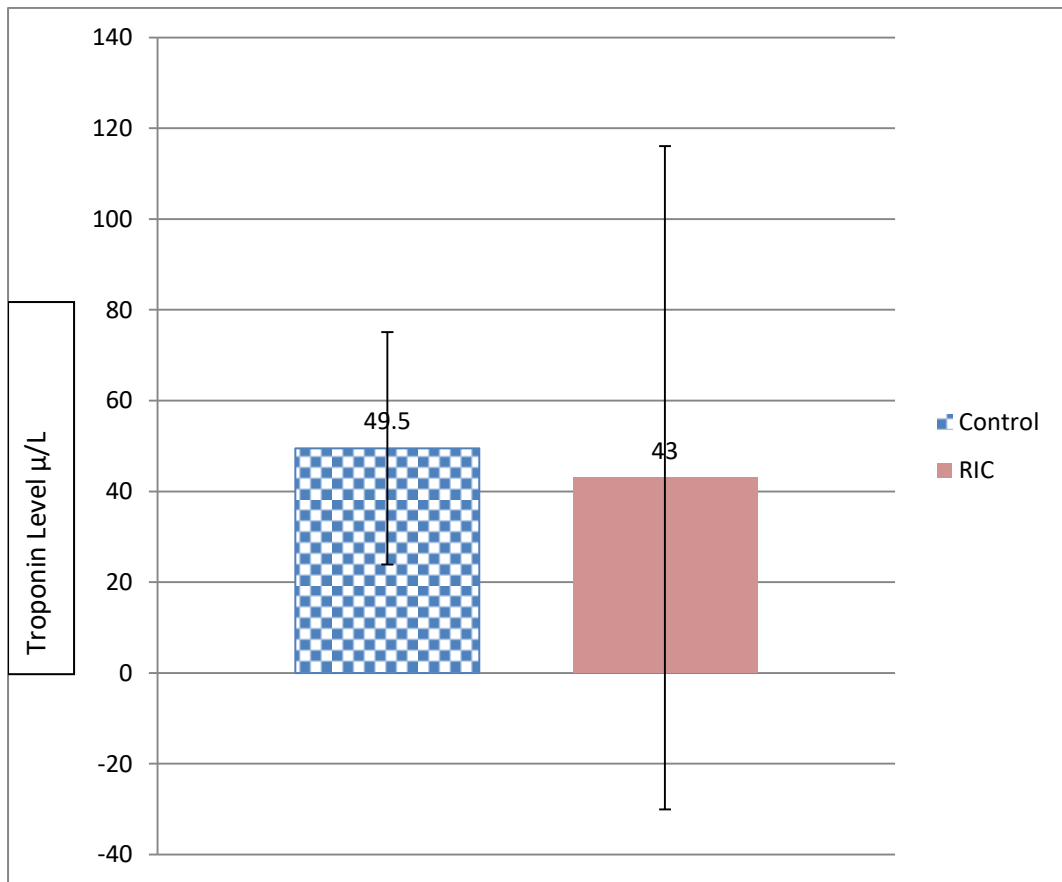
CK-MB assay at St Thomas' Hospital measured the CK-MB mass, using the unit of measurement of microgram per litre ( $\mu\text{g/l}$ ). The reference range was different in men and women: 0.1-4.94  $\mu\text{g/l}$  in males and 0.1- 2.88  $\mu\text{g/l}$  in females.

As seen in the graph below, at 6, 12 and 24 hours post PCI, the CK-BM level was lower in the RIPC group compared with the control group. This attenuation was not statistically significant. P values, 0.61, 0.67, and P 0.47 for 6, 12 and 24 hour differences respectively.

**Graph 4.5 CK-MB Level Over 24 hrs Post PCI- St Thomas' Hospital**



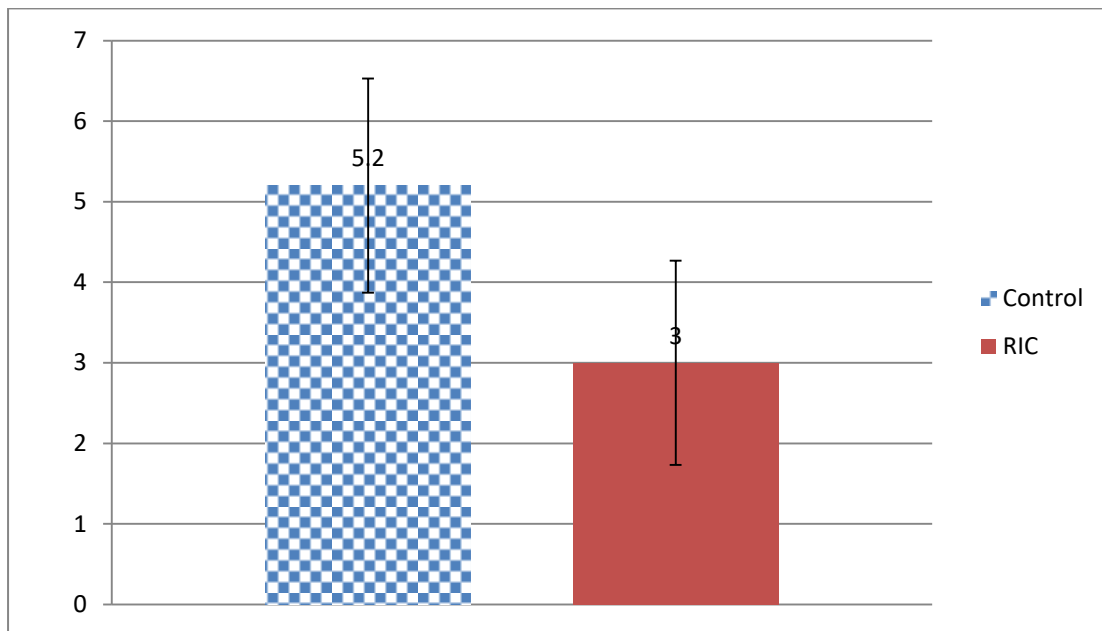
**Graph 4.6 Troponin level 24 hr post PCI-Rotablation Group**



***P = 0.8***

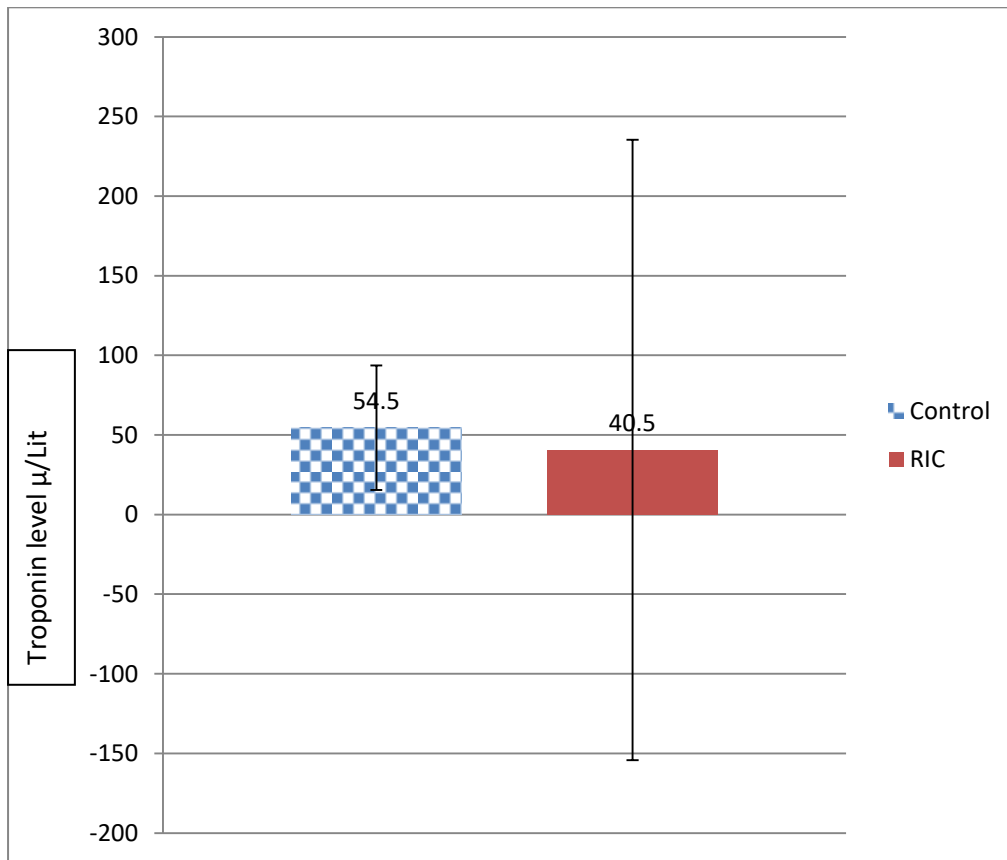


**Graph 4.7 CK-MB Level 24 hr post-PCI-Rotablation Group –STH**



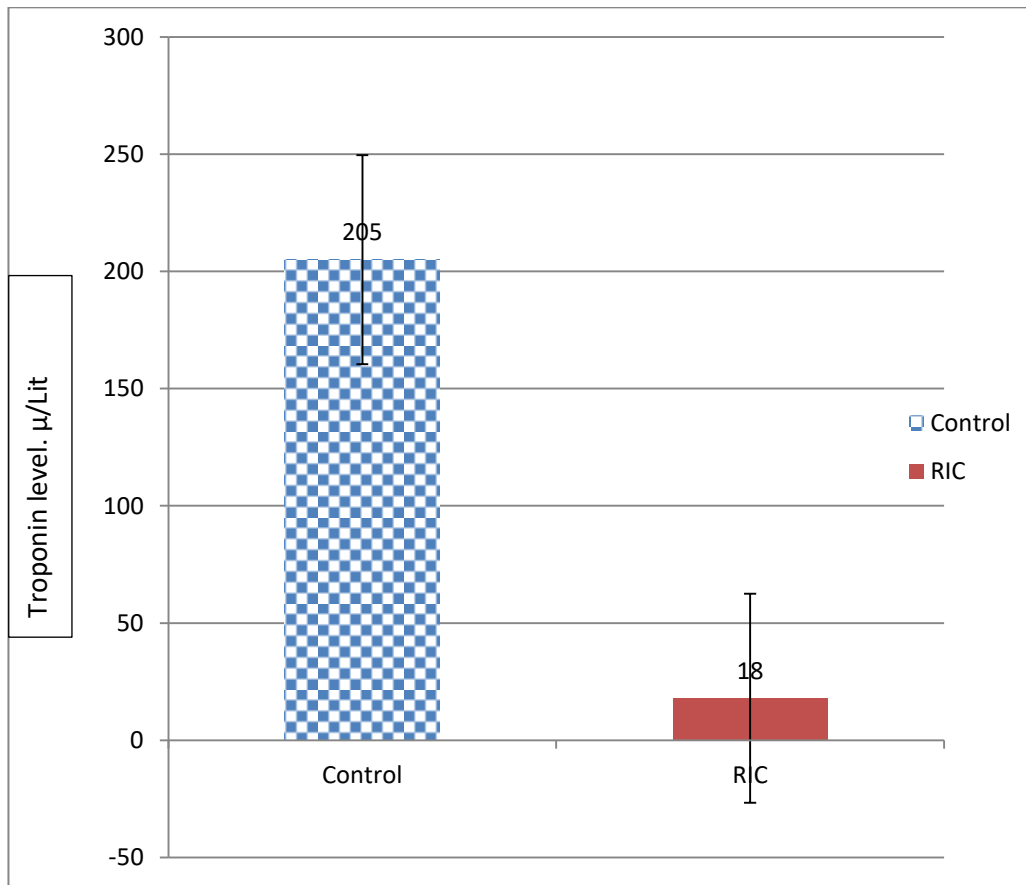
***P = 0.1***

**Graph 4.8 Troponin level 24 hr post PCI-CTO group**



***P = 1.0***

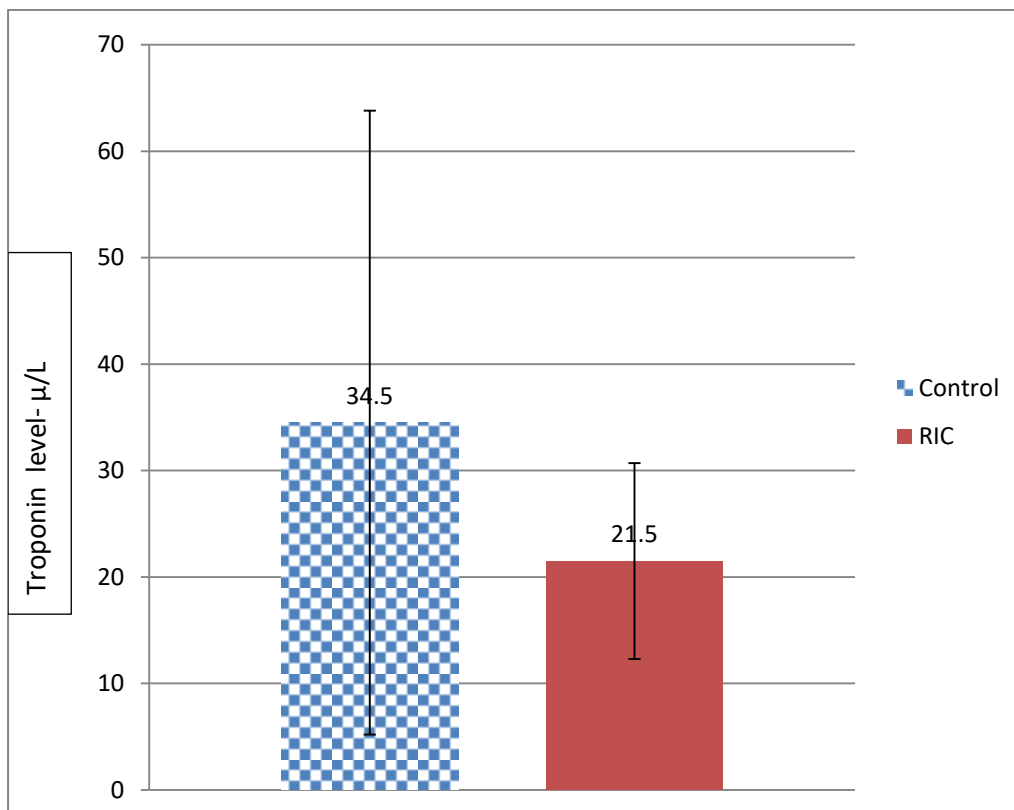
**Graph 4.9 Troponin level 24 hr post PCI- Syntax>23**



**$P = 0.2$**

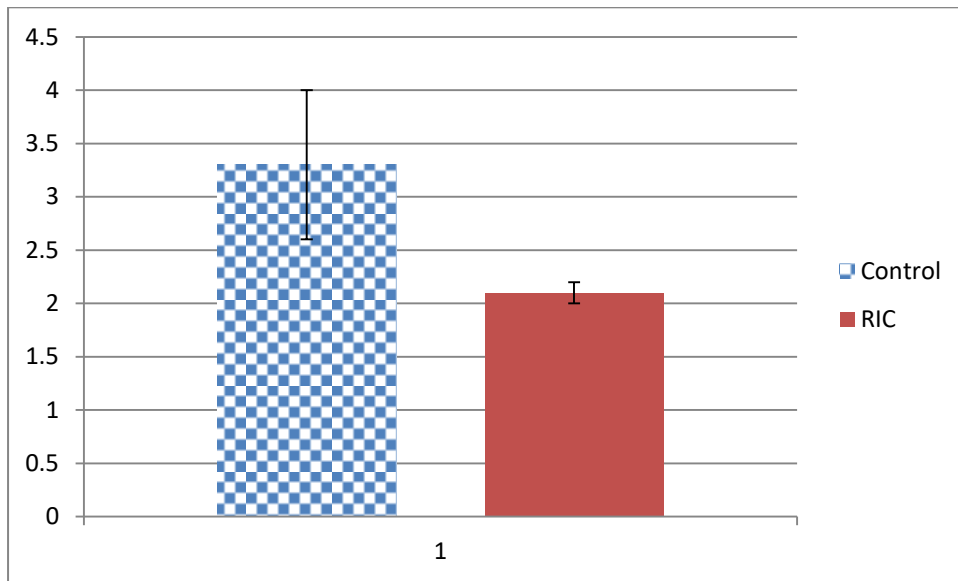
There was no type A lesions in this group.

**Graph 4.10 Troponin Level 24hr post PCI -Vein graft/ Laser**



**$P = 0.6$**

**Graph 4.11 CK-MB Level 24hr post PCI – Vein graft and Laser**



***P= 0.5***

**Table 4.6 Troponin Level\* & Procedure Time\* in Each Group**

<b>Group</b>	<b>Procedure Time (min)</b>	<b>Troponin Level ng/Lit</b>
CTO	55.1	54.5
Rotablation	50.5	43
Syntax >23	33.6	27
PCI to VG and Laser	44.1	26

\*Median Troponin level and mean procedure time.

## **Secondary Endpoint- Ischaemic ECG Changes**

In the ERIC-PCI trial, seven patients developed ST segment changes and arrhythmia during PCI. Ventricular tachycardia (VT) and ventricular fibrillation (VF) occurred in two patients in the control group and in one patient in the RIPC group. ST elevation during PCI procedure was observed in one patient in the control group and in two patients in the RIPC group. ST segment depression developed in one patient in the RIPC group. There was no statistically significant difference between these two groups in these characteristics,  $P = 0.8$ .

## **Secondary Endpoint- Reduced peri-PCI injury in CMR**

For identification of possible PCI-induced myocardial oedema and new late gadolinium enhancement (LGE), two CMR scans were planned for each patient- if there were no contraindications to CMR. First scan, prior to the elective PCI and the second scan, within 7 days post PCI. Nineteen patients, who had no contraindications, underwent pre-PCI cardiac scan at Great Ormond Street Hospital. Of these, 17 underwent the second CMR 2-7 days post PCI. The main researcher scanned all patients, apart from one occasion when due to commitment at another hospital, the CMR was performed by a radiographer.

In March 2012, following an interim assessment of the cardiac MRI images by expert specialist, it was decided to abandon CMR investigations. No new infarction or myocardial oedema was noted in the images of the seventeen patients who had CMR post PCI, even when Troponin level 24 hours post PCI was elevated 5 times higher than baseline. Hence, CMR investigation was not continued any further as it was deemed futile.

Among the group of patients who underwent CMR pre and post PCI, the median Troponin level was 25ng/Lit. Among patients who underwent CMR, pre and post PCI, in 2 patients only the Troponin level was significantly elevated without any evidence of oedema or fibrosis on CMR.



**Table 4.7- CMR analysis**

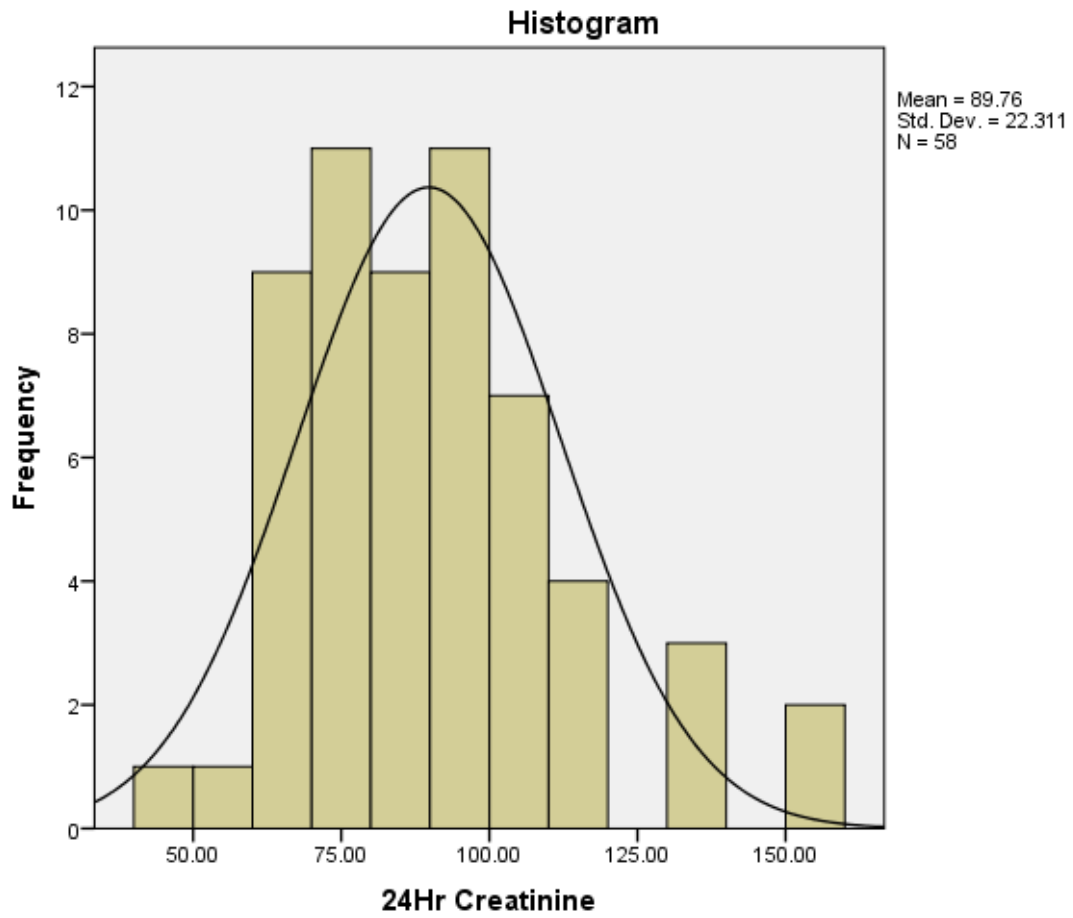
<b>Demographics</b>	<b>Control</b>	<b>RIPC</b>	<b>P value</b>
Troponin Level (ng/L)	48	11.5	0.09
LVEDV(mls)	153±26.2	160.8±54.8	0.36
LVESV(mls)	63.3±31.2	79.2±65.3	0.28
SV (mls)	89.7±14.4	84.4±15.2	0.44
Ejection Fraction (%)	55.1±16.9	64.85±17.0	0.53
Myocardial oedema	Nil	Nil	
New LGE	Nil	Nil	
New RWMA	Nil	Nil	

## **Secondary Endpoint- Reduction of CI-AKI**

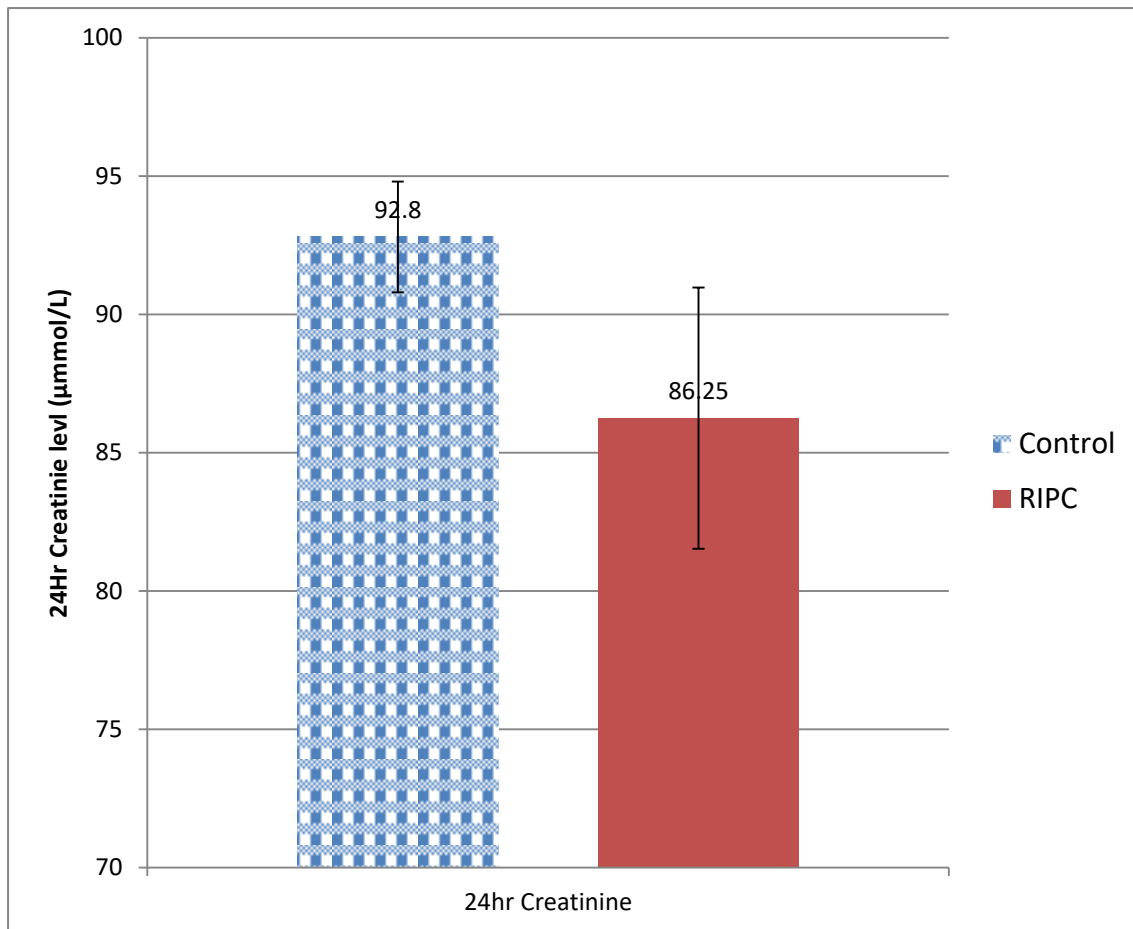
CI-AKI is defined as either an absolute increase in serum creatinine (Cr) concentration of 44.2  $\mu\text{mol/l}$  (or 0.5 mg/dl) or a 25% relative increase of serum creatinine from baseline. <sup>(332, 331)</sup> In the ERIC-PCI trial, creatinine levels were checked at times zero (pre-PCI) and 6, 12, 24 hours post PCI. In the control group, the mean of creatinine level pre-PCI was 89.6 ( $\mu\text{mol/l}$ ). Twenty-four hours later, this value increased to 92.8 ( $\mu\text{mol/l}$ ). In the RIPC group, the mean creatinine level was 85.7 ( $\mu\text{mol/l}$ ) at time zero (pre-PCI) and 86.2( $\mu\text{mol/L}$ ) 24 hours post PCI, therefore by definition there was no evidence of CI-AKI in each group. Patients did not receive any n-acetylcysteine or sodium bicarbonate prior to PCI. Ramipril was discontinued only in Basildon centre.

As discussed in Chapter 1, one of the most promising renal biomarkers includes NGAL. In a recent meta-analysis by Haase et al, it was confirmed that NGAL is a valuable renal biomarker in all settings of AKI investigated. <sup>(362)</sup>

In the ERIC-PCI trial, NGAL samples were collected in Ethylene Diamine Tetra Acetic Acid (EDTA) bottles before PCI and at 6, 12 and 24 hours post PCI. The samples were spun, centrifuged, plasma aliquoted and stored in -20 degree centigrade in the laboratories of the recruiting centres. The final analysis of NGAL samples in the core laboratory at UCLH could not take place since 48-hr creatinine levels were lacking. Creatinine level 48 hours post an insult to kidneys, is a prerequisite of NGAL analysis. Due to logistic issues, patients could not remain inpatient for 48 hours post PCI.

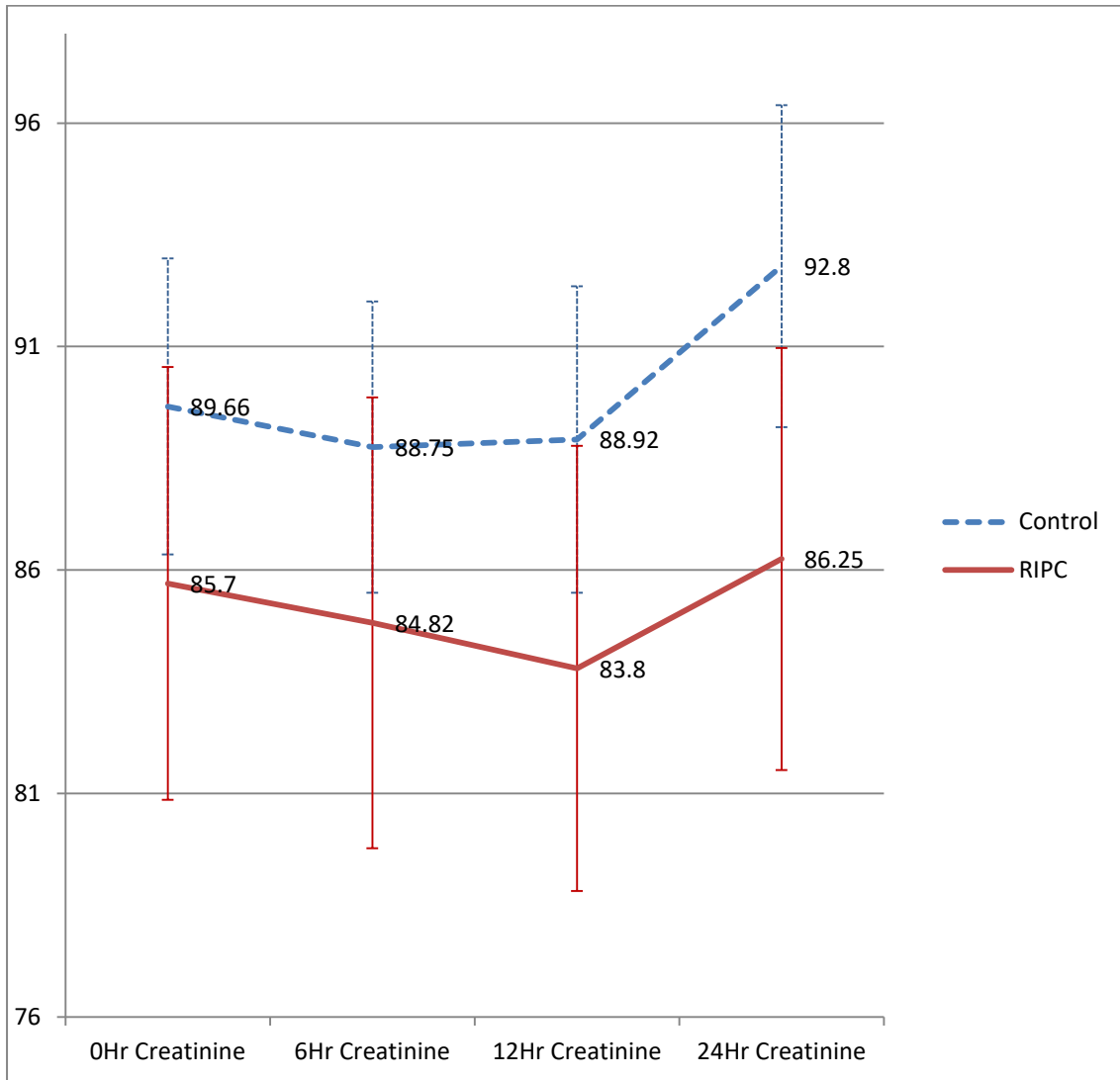


**Graph 4.12 Creatinine Level 24 Hours post PCI**

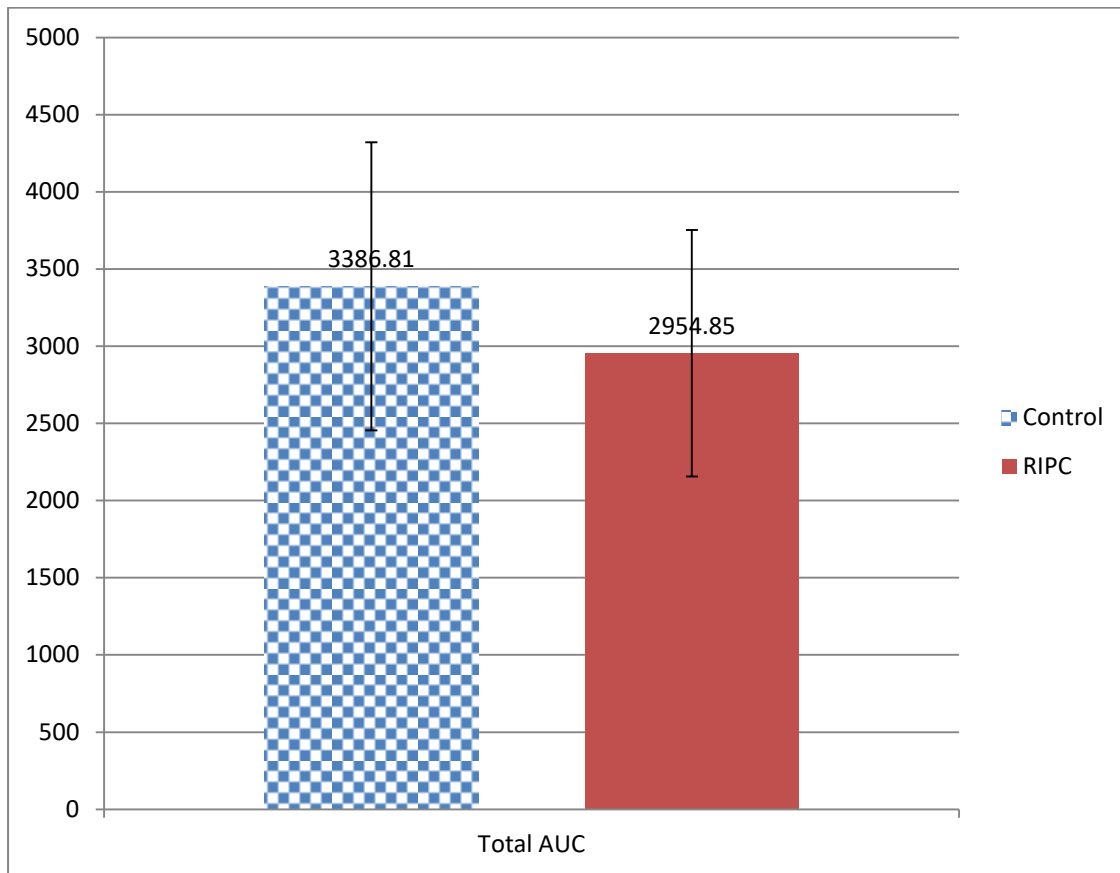


***P = 0.26***

**Graph 4.13 Creatinine Levels over 24 Hours Post PCI**



**Graph 4.14 Creatinine total Area under the Curve (AUC)**



**P = 0.3**

## **Secondary Endpoint- 30 day MACE**

The follow-up data were obtained from a clinical interview over telephone by the main researcher who was not blinded to the group allocations. Within 30 days post complex PCI, none of the patients had experienced major cardiovascular event, revascularisation, re-admission to hospital or death from cardiovascular causes. No participants were lost to follow up.

## **Adverse Outcome**

Inflation of a blood pressure cuff up to 200 mg Hg for 5 minutes to induce transient limb ischaemia is harmless and simple, not associated with any major adverse events. During the course of the ERIC-PCI study however, an adverse event occurred which was reported to the relevant R&D and ethics departments. The incident was documented in the medical notes and case report form (CRF). In this regard, discolouration of the right upper arm happened following inflation of the blood pressure cuff up to 200 mmHg for 5 minutes x 4 times (*Figure 5.1*). This adverse effect, following the use of blood pressure cuff on the upper or lower arm to induce remote ischaemic preconditioning, has not been reported before. No other vascular or neurological components were associated with this event. The discolouration resolved in less than 24 hours without any vascular or neurological sequels.



*Figure 5.1-* Discolouration of the right upper limb following inflation of the BP cuff up to 200 mmHg



# **CHAPTER 5**

## **DISCUSSION**

Coronary heart disease is one of the leading causes of death and disability worldwide, resulting in an estimated 7.3 million deaths per year.<sup>(1)</sup> Revascularisation of coronary arteries with PCI has become the treatment of choice for most of the patients with CHD, with a rapidly growing prevalence over the last few years. Despite advances in PCI technique and introduction of anti-proliferative medications in drug eluting stents, PCI in patients with stable coronary heart disease has failed to show any clear improvement in the prognosis of heart disease over and above that already achieved by medical therapy.<sup>(408,409)</sup> This may at least, in part, be explained by the myocardial damage caused by PCI; peri-procedural myocardial injury or type 4a MI<sup>(3)</sup> as evidenced by troponin and CK-MB rise in 10-30% of cases.<sup>(9, 20, 410)</sup>

PCI-related myocardial injury, even with no immediate clinical presentation has a poor impact on prognosis of patients with coronary heart disease.<sup>(9-12, 24, 411)</sup> Clinical outcomes after PCI with very high procedural CK-MB levels (>5 x or >8 x the upper limit of normal) have prognostic implications similar to those of spontaneous acute MI.<sup>(46, 412, 413)</sup>

In the PPCI setting, reperfusion injury and in the elective PCI setting, microembolisation of the plaque debris in the distal vasculature, inflammation causing microvascular obstruction, myocardial oedema, slow/no-flow, coronary dissection and side-branch occlusion have been proposed as the most likely mechanisms of myocardial injury.<sup>(15, 20, 29, 32)</sup> Given the strong correlation between PCI-related myocardial injury and clinical outcomes, a significant amount of research has been performed to try to understand the pathophysiology behind this injury and to find potential ways of protecting the heart in the PCI setting whether achieved by drugs, devices, or technique. To date, no pharmacological therapies have been successfully developed to entirely protect the myocardium from ischaemic injury.

Some of these strategies seek to stimulate the intrinsic mechanism of cell protection based on the concept of ischaemic preconditioning. RIPC, which refers to transient episodes of remote

organ (limb in most studies) ischaemia prior to cardiac ischaemia, is a phenomenon which could potentially offer cardioprotection and significantly improve practice. The RIPC concept has been evolved from an experiment which showed brief and repeated episodes of ischaemia applied in circumflex artery, followed by 1 hour sustained occlusion in the left anterior descending artery, resulted in significant reduction in infarct size in the preconditioned group compared to the control group which underwent one hour sustained occlusion of the LAD without preconditioning.<sup>(187)</sup> Since then, this model has been widely studied and reproduced. A large number of RIPC studies have shown promising results in the experimental laboratory settings and our understanding of the basic science has improved significantly but many challenges have been encountered in translating the message implicit in the experimental models of ischemic preconditioning to a clinical application.

In clinical practice, the exact benefit of RIPC in protecting the human myocardium in elective PCI and CABG settings has been elusive with inconsistent results so far. Laboratory findings have appeared promising but translating cardioprotection from the laboratories to the bedside has proved challenging and little practical success has been realised.<sup>(414)</sup> Clinical findings in PCI and CABG have also failed to show consistent cardioprotective outcomes.

In elective PCI settings, some proof of concept studies, have shown significant reduction in Troponin release and infarction size has been evident whilst some others have failed to prove benefits. (Table 5.1) In the first detailed coronary hemodynamic study of this model of controlled coronary occlusion in man, Deutsch et al. showed that two 90-second balloon occlusions separated by a 5-minute period of reperfusion, resulted in statistically significant reductions in objectively assessed angina score, ST-segment–elevation, left ventricle filling pressure increase, regional coronary blood flow, and lactate release from the coronary circulation.

Similar conflicting results have been observed in some clinical trials in the cardiac surgery setting. Whilst a few studies showed RIPC is effective in reducing procedural-related myocardial injury and improving cardiovascular outcomes,<sup>(226, 416, 234)</sup> the results of two recent adequately powered large randomised controlled trials, ERICCA (The Effect of Remote Ischemic Preconditioning on Clinical Outcomes in Patients Undergoing Coronary Artery Bypass Surgery)<sup>(222)</sup> and RIPHeart ( The Remote Ischaemic Preconditioning for Heart Surgery )<sup>(223)</sup> were disappointing without showing benefit from RIPC in reducing CABG related myocardial and kidney injuries, MACE or perioperative myocardial injury, in 1612 and 1385 patients respectively. Disappointments observed in clinical trials are usually due to small sample sizes and potential confounding variables such as age, cross-clamp time, co-morbidities and co-medications. ERICCA and RIPHeart however were not small studies. Therefore, the most reasonable way to explain the disappointing results of ERICCA<sup>(222)</sup> and RIPHeart<sup>(223)</sup> trials, apart from the fact that RIPC is less effective in aged and diabetic hearts, is probably the use of propofol and volatile anaesthetics as the primary anaesthetic agents in these large well-powered trials. Propofol and volatile anaesthetics are known to reduce/inhibit the cardioprotective effect of RIPC.<sup>(417)</sup> In the ERICCA trial, anaesthesia was not standardised although almost 90% of patients received propofol. In the RIPHeart trial, anaesthesia was standardised and performed with intravenous propofol in all cases. Also cardiopulmonary bypass itself as well as hypothermia and cardioplegia can be cardioprotective per se.<sup>(418)</sup>

With regard to PCI, Iliodromitis et al. performed one of the first studies that assessed the role of RIPC in this setting in 2006.<sup>(220)</sup> The results of this randomised controlled trial that used three 5-minute cycles of inflation and deflation of blood pressure cuff showed that circulating CRP levels increased within 48 hours after PCI and RIPC could not prevent this. Furthermore, RIPC was

associated with a worse increase in cardiac enzymes and troponin I release, even after uncomplicated single-vessel angioplasty.<sup>(220)</sup> Interestingly there seemed to be an enhanced inflammatory response after RIPC in the absence of statin treatment, which conferred a benefit in this respect.

Following this discouraging outcome, Hoole et al.<sup>(15)</sup> in 2009 investigated the effect of RIPC in 202 patients undergoing elective simple PCI. The CRISP study used a protocol of RIPC with three 5-min cycles of inflation and deflation of a blood pressure cuff. Patients who received this protocol experienced less chest pain during the angioplasty, less ST segment deviation and a lower 24-hour troponin I release. The median cTnI at 24 hours after PCI was lower in the RIPC group compared with the control group (0.06 versus 0.16 ng/ml;  $P= 0.040$ ). After RIPC, cTnI was  $< 0.04$  ng/ml in 44 patients (42%) compared with 24 patients in the control group (24%);  $P= 0.01$ . In 2013, the CRISP Stent investigators published 6-year outcome of recruited patients, showing both long-term MACE benefits,<sup>(215)</sup> an outcome that may be considered mysterious considering the fact that remote conditioning stimulus should not last for such a long period of time.

It was in 2010 when Botker et al. demonstrated the potential for pre-hospital use of RIPC in the setting of acute MI (4 cycles of 5-minute upper limb cuff inflation and deflation, delivered in the ambulance).<sup>(96)</sup> In a trial of 333 patients, an improvement in myocardial salvage index (%) at 20 days after PPCI was demonstrated in the group randomised to receive preconditioning.<sup>(96)</sup> Same investigators<sup>(210)</sup> published a 3.8-year outcome of recruited patients which showed RIPC before PPCI seemed to have improved long-term clinical outcomes in patients with STEMI, an outcome which again appears a mysterious effect of RIPC.

Following the findings by Hoole et al. and Botker et al, Prasad et al. in 2013 published the results of a trial that sought to determine the efficacy of RIPC in elective PCI setting.<sup>(215)</sup> Ninety-five

patients with both stable and unstable angina were enrolled into this study. The protocol used in this trial was three 3-min cycles of inflation and deflation of blood pressure cuff, immediately preceding PCI to minimise the delay between angiography and PCI. There was no difference in the primary endpoint of the frequency of PCI related myocardial injury which occurred in 22 (47%) and 19 (40%) patients in the RIPC and control groups, respectively,  $P = 0.42$ . There was significant increase in CRP post-PCI in both groups ( $P < 0.001$ ).

The discrepancy between the results of the study performed by Hoole et al.<sup>(15)</sup> and the one performed by Prasad et al.<sup>(215)</sup> is perhaps due to recruitment of patients with unstable angina in Prasad's study whereas in CRISP study stable patients undergoing simple PCI were involved. Furthermore, the intervention protocol also was different between the two studies. Three 5-min ischaemia/reperfusion in the CRISP study versus three 3-minutes in Prasad's study- an ischaemic period that may not have been adequate to condition. This however could be a subject of debate as some studies have reported cardioprotective effects with only one cycle of RIPC.<sup>(207,209)</sup> In addition, in the study by Prasad et al. the preconditioning intervention was administered immediately before PCI. Although no optimal time has yet been established between the preconditioning and the angioplasty, the stimulus might have been applied too soon.

Table 5.1 summarises all RIPC trials since 2006 in PCI setting.

**Table 5.1 RIPC clinical trials in PCI setting**

Year	N	Author	Clinical setting	Intervention	Outcome
2017		Yilmaztepe <sup>(207)</sup>	Elective PCI	Upper arm 1 x 5 min	Lower Trop I in RIPC group
2017		Ladejobi <sup>(208)</sup>	PPCI	Upper arm 4 x 5 min	Reduced serum BNP and HF
2014		Zografos <sup>(209)</sup>	Ad hoc PCI	Upper limb 1 x 5 min	Lower Trop I in RIPC group
2014		Sloth <sup>(210)</sup>	PPCI	Upper limb 4 x 5 min	Lower MACCE in RIPC group
2014		Liu <sup>(211)</sup>	Elective PCI	Upper limb 3 x 5 min	Lower Trop I, CK, CK-MB in RIPC group
2014		Manchurov <sup>(212)</sup>	PPCI	Upper limb 4 X 5 min	Improved endothelial function
2013		Davies <sup>(213)</sup> (CRISP Stent f/u)	Elective PCI	Arm 3 x 5 min	MACCE-free survival both short- and long-term f/u
2013		Ahmed <sup>(214)</sup>	Elective PCI	Arm 3 x 5 min	Lower Trop-T in RIPC group
2013		Prasad <sup>(215)</sup>	Elective PCI	Arm 3 x 3 min	No difference in Trop T release in sham and RIPC groups
2012		Ghaemian <sup>(216)</sup>	Elective PCI	Lower limb 2 x 5 min	Lower Troponin T in RIPC group
2012		Luo <sup>(217)</sup>	Elective PCI	Arm 3 x 5 min	Lower Troponin I in RIPC group
2011		Munk <sup>(218)</sup>	PPCI	Arm 4 x 5 min	Non significant improvement in LV Systolic function in RIPC group
2010		Botker <sup>(96)</sup>	PPCI	Arm 4 x 5 min	Increased myocardial salvage index in RIPC group
2010		Rentoukas <sup>(219)</sup>	PPCI	Arm 3 x 4 min plus morphine	Full ST segment resolution in RIPC plus morphine group
2009		Hoole <sup>(15)</sup>	Elective PCI	Arm 3 x 5 min	Lower Troponin I in RIPC group
2006		Iliodromitis <sup>(220)</sup>	Elective PCI	Both arms 3 x 5 min	Increased Top-I, CK-MB and CRP in RIPC group

**The ERIC-PCI trial** was the first study that assessed the efficacy of RIPC in the setting of complex coronary anatomy and difficult PCI, defined as severely calcified or occluded vessels requiring laser and rotablation , and SYNTAX score between 23 and 35 . Myocardial injury and subsequent troponin release related to coronary intervention is reportedly higher in this so-called 'complex' procedures.

In this multicentre randomised controlled trial running from April 2011 until August 2013, RIPC constituting of 4 cycles of 5-minute ischaemia/reperfusion on upper arm, appeared to have a neutral protective effect on myocardial injury associated with complex PCI. The primary outcome measure in the ERIC-PCI trial was the incidence and extent of PCI –related myocardial injury as assessed by serum cardiac biomarkers at times 6, 12 and 24 hours post PCI. The reference interval of hs Troponin was 0-14 ng/l.

The median 24-hour Troponin T level was 48 ng/l in the control group and 32.5 ng/l in the RIC group. Although the Troponin release at 24 hours post PCI appears to be attenuated in the preconditioned group, this difference was not statistically significant, P =0.39.

Likewise, the incidence of PCI-related myocardial injury 24 hours post-PCI was not significantly reduced. Significant rise of the Troponin level 24 hours post PCI was observed in 46.9% of patients in the control group and in 26.7 % of patients in the RIPC group. This difference was not statistically significant, P= 0.12.

According to the previous definition of MI in 2007, significant Troponin release (3 times higher than baseline) was observed in 58.1% of the control group and 42.3% of the RIC group, P value 0.3



Although the ERIC-PCI trial RIPC did not demonstrate significant reduction in Troponin release in patients undergoing complex PCI, in general, the results appear to favour the hypothesis and there is a trend towards efficacy of RIPC in reducing Troponin release peri-PCI.

The ERIC-PCI was an underpowered study. This fact is probably the main reason for the lack of statistically significant findings but similar to some other neutral or negative trials, a few obstacles and confounding factors potentially influenced the efficacy of RIPC. The most important issue facing clinical preconditioning studies is that the experimental studies are mostly done in young and healthy animals. Lack of advanced age, comorbidities such as dyslipidaemia, hypertension and medications (mainly P2Y12 inhibitors, statins, antidiabetics, beta-blockers, angiotensin converting enzyme inhibitors, angiotensin receptor 1-antagonists, etc.) may confound the translation of cardioprotection from experimental animal studies to clinical practice and interfere with the protective effect of remote ischaemic conditioning. <sup>(422, 423)</sup>

Experimental studies using human atrial muscle from patients undergoing CABG, from aged and diabetic patients and patients with heart failure have confirmed the role of comorbidities on the conditioning threshold and have demonstrated resistance to various conditioning strategies. <sup>(317, 419,420)</sup> It is known that the power of cardioprotection is lost or limited in these conditions probably due to reduction of cardioprotection signalling proteins which results in an increase of the threshold to achieve cardioprotection. <sup>(324, 327, 421)</sup>

Understanding the influence of confounders is essential towards translation of laboratory achievements to clinical practice and towards understanding the obstacles that have negative influence on efficacy of RIPC in elective revascularisation settings (elective PCI and CABG) as well as current trial, the ERIC-PCI. Development of rational therapeutic approaches to protect the

ischaemic heart requires preclinical studies that examine cardioprotection specifically in relation to cardiovascular risk factors and their medications. <sup>(422)</sup>

Confounding variables are explained in details below.

## **Confounding Variables- Sample Size**

Firstly and more importantly, our study was underpowered for the primary end point of 20% absolute reduction in the incidence of PCI-related myocardial injury, with 80% power and significance of 0.05. When calculating power, it was estimated that 100 patients in each group were required to reach the 80% power for this study. Due to the high efficacy of cardiovascular medications- mainly statins- complex coronary lesions are not as prevalent as previous years. Patients with significant multi-vessel coronary artery disease and SYNTAX score > than 35 will benefit from CABG more than PCI. Hence, patients with complex coronary artery disease and SYNTAX score > 35 were offered cardiac surgery. More importantly, complex PCI requires special skills, technique and devices, which were mainly available at one recruiting centre, St Thomas' Hospital, London. Therefore, due to the above limitations and logistic issues, recruiting 100 patients in each arm (200 in total) within 2 years was not feasible. In total, 88 patients were recruited into the ERIC-PCI study. Significance might have been demonstrated if the sample size were bigger.

## Confounders- RIPC stimulus

The optimal RIPC stimulus remains unclear but a threshold stimulus must be reached in order to achieve protection. In most of the clinical studies the beneficial effect has been obtained by 3 or 5 cycles of 5-minute blood pressure cuff inflation/deflation on the upper limb, initiated either at the time of reperfusion or 1-3 hours prior to PCI. (Table 5.1) There is an argument that in ischaemic preconditioning (and, possibly, remote preconditioning), prolongation of the time interval between the brief ischaemic stimulus and the onset of sustained ischaemia to a period of more than 4 hrs results in a loss in cardioprotective efficacy, while a further extension to 12 to 24 hrs initiates a second and distinct, delayed or late phase of protection that persists for around 3 to 4 days.

A recent clinical study has suggested that one cycle of 5-minute blood pressure cuff inflation/deflation could also induce protection during elective PCI. <sup>(209)</sup>

The first window of protection lasts for 2 to 3 hours and the onset appears to be instant, as the RIPC initiated immediately prior to revascularisation also has reduced infarct size in STEMI patients. <sup>(420, 425)</sup>

In the ERIC-PCI trial, 4 cycles of 5-min BP cuff inflation and deflation were applied which should be an ideal stimulus. It was ensured that the preconditioning stimulus was applied in no longer than 3 hours prior to PCI.

## Confounding Variables- Demographics and Risk Factors

Coronary heart disease in humans is associated with cardiovascular risk factors and comorbidities, including hypertension, metabolic disease such as dyslipidaemia, diabetes, insulin resistance, and obesity. In addition aging is another major risk factor for development of ischaemic heart disease. Following original observation of loss of preconditioning effect in hyperlipidaemic rodents in 1995,<sup>(327)</sup> it has been well established that risk factors for coronary heart disease interfere with cardioprotection. They induce fundamental alterations in cellular signalling cascades that affect the development of ischaemia/reperfusion injury per se and responses to cardioprotective interventions.<sup>(422)</sup>

Patients' demographics were evenly distributed in the ERIC-PCI trial.

### Age and gender

Age is an important confounding factor in translation of the cardioprotection induced by RIPC to clinical practice. It is well known that myocardium can become resistant to ischaemic preconditioning and also postconditioning with age,<sup>(315, 320,426)</sup> notably through reduced expression of important signalling proteins.<sup>(321)</sup> In a study by den Munckhof et al. with the endpoint of endothelial function rather than myocardial infarct size, increased age was associated with loss of protection by ischaemic preconditioning against endothelial dysfunction after ischaemia/reperfusion in the brachial artery.<sup>(427)</sup> Most of the successful laboratory studies were performed in young adult rats and mice (aged 3–4 months) which are equivalent to the human age of 7–10 years.<sup>(314, 321)</sup>

The patients' demographics have changed over the last few years with more patients older than 75 years at the time of elective revascularisation, with more co-morbidities. As well as age, the female gender also affects cardioprotection conferred by preconditioning. Experimental and clinical studies <sup>(428,429)</sup> confirm that female hearts have an increased resistance to ischaemia/reperfusion injury, associated with an altered distribution of PKC and extracellular signal-regulated kinase (ERK) isoforms compared with male hearts. <sup>(430)</sup>

Most of the successful laboratory studies have been performed in young and healthy animals, whereas the average age of coronary heart disease is between 50 to 60 years.

In the ERIC-PCI study, the mean age of all recruited patients was 66.9 years with no significant difference between the two groups.

## **Dyslipidaemia**

Although some conflicting results have been reported so far, most of the preclinical and clinical studies have shown that hyperlipidaemia per se and not necessarily atherosclerosis, leads to a significant aggravation of myocardial ischaemia/reperfusion injury and attenuates the cardioprotective effect of preconditioning. <sup>(426)</sup> Expansion of infarct size in a hyperlipidaemic pig model was shown by Osipov et al. <sup>(431)</sup> The loss of the infarct size limiting effect of ischaemic preconditioning <sup>(432, 433)</sup> and late ischaemic preconditioning ) have been shown in different models of diet-induced hyperlipidaemia in rats.

The mechanism by which hyperlipidaemia may influence the severity of myocardial ischaemia/reperfusion injury and reduces cardioprotection is not fully understood but is well accepted that hyperlipidaemia can induce changes in cardioprotective signalling pathways. Statins can activate the mitochondrial K-ATP channel; thereby triggering ischaemic

preconditioning. <sup>(329)</sup> Besides plaque stabilisation, statins can improve endothelial function and have been shown to have anti-inflammatory characteristics and reduce thrombogenic response. <sup>(327,410)</sup> Thus, dyslipidaemia could be another confounding factor in our study. In terms of prevalence of dyslipidaemia, the two groups were well balanced. Statins are effective through different mechanisms other than lipid lowering.

## Diabetes

Diabetes mellitus is a major risk factor for CHD with an increasing incidence and a negative prognosis in patients who undergo PCI. <sup>(434)</sup> Diabetic heart is more susceptible to acute myocardial ischaemia/reperfusion injury and seems to behave differently to the phenomena of conditioning. <sup>(314,414)</sup> Much debate surrounds the benefit of ischaemic conditioning in diabetic patients. <sup>(435)</sup> It is thought that diabetes may limit the activation of prosurvival cellular mechanisms against ischaemia and may interfere with the cardioprotective mechanisms, attenuating the effectiveness of these therapeutic strategies. Although a diabetic myocardium can be protected by ischaemic preconditioning the threshold required to achieve this protection is higher than normal non-diabetic hearts. <sup>(324)</sup> The majority of studies show that the presence of diabetes mellitus may affect the mechanisms for cell protection against ischaemia, reducing the protective effect attributed to ischaemic conditioning. <sup>(435)</sup> A variety of different mechanisms have been suggested to contribute to the impaired response of the diabetic heart to preconditioning. These include impaired activation of known intracellular prosurvival signalling pathways, such as the Akt and ERK1/2 components of the RISK pathway. <sup>(301)</sup>

An important limitation concerning the applicability of the results of experimental animal studies to humans is the frequent presence of diabetes in humans.

In the ERIC-PCI trial, on average, one third of patients in each group were diabetic. More than 80% of patients in each group were hypertensive.

## **Diseased and Heterogeneous Coronary Arteries**

Experimental studies on myocardial ischaemia/reperfusion injury and ischaemic preconditioning are usually performed in healthy young animals with virgin coronary circulation. In real clinical situation, atherosclerosis develops progressively over time. Atherosclerotic plaque rupture in the coronary artery with superimposed intraluminal thrombotic occlusion of the arterial lumen is the culprit in acute MI setting. In an acute event, plaque rupture superimposes on the underlying atherosclerosis, further complicated by intraluminal platelet aggregation and coagulation. In contrast, experimental studies usually rely on abrupt closure and reopening of a young and healthy epicardial coronary artery with external devices. Therefore, the status of both the epicardial coronary arteries and the coronary microcirculation is vastly different between clinical reality and most experimental models.<sup>(420)</sup> These differences should be considered as confounders in translation of cardioprotective strategies.<sup>(420)</sup> In fact, in most but not all of the more clinically relevant conditions, a diseased coronary circulation tends to attenuate the efficacy of cardioprotection.

## **Confounding Variables- Concomitant Medical Therapy**

Medications commonly taken by patients with cardiovascular disease are major confounders and have an influence on novel cardioprotective strategies such as RIPC, either by mimicking the

protection of RIPC or by abolishing the effect of RIPC. Anti-platelets, ACE inhibitors, beta-blockers, Nicorandil, Nitrates, statins, beta-blockers and diabetes medications are protective and may inadvertently precondition the myocardium. <sup>(314)</sup> and have each been demonstrated to reduce infarct size in the laboratory settings, by recruiting preconditioning pathways. <sup>(436)</sup>

## **Anti-platelets- P2Y12 inhibitors**

P2Y12 platelet antagonists which are now standard of care for the treatment of coronary heart disease, have intrinsic cardioprotective properties, similar to preconditioning, independent of their effects on platelet aggregation. Nevertheless, the anti-ischaemic role of P2Y12 inhibitors is mainly due to their anti-platelet activity. <sup>(145)</sup> P2Y12 inhibitors have demonstrated significant improvements in cardiovascular mortality and are the main adjunctive treatment with PCI. P2Y12 inhibitors-induced protection depends on similar signalling components as conditioning. Pre- PCI clopidogrel is known to decrease procedural related ischaemic events in observational registries. <sup>(384)</sup> Ticagrelor also inhibits adenosine re-uptake via the equilibrative nucleoside transporter, increasing adenosine and potentially triggering conditioning via this route. <sup>(437)</sup>

Although administration of Aspirin is also mandatory in coronary heart disease, there is no evidence to suggest that aspirin is cardioprotective. <sup>(438)</sup>

Unless already on long term treatment, all patients in the ERIC-PCI trial received loading dose of clopidogrel on the day of elective PCI.

It is therefore likely that the effect of RIPC could have been attenuated by the use of above medications.



## Antidiabetics

Antidiabetics may impact on the cardioprotective efficacy of conditioning strategies by modulating the underlying intracellular signalling pathways within the heart in two major ways. The antidiabetic therapy may either interfere with the cardioprotective signalling pathway, thereby blocking the "conditioning" strategy, or it may mimic the "conditioning" strategy, thereby inducing cardioprotection.<sup>(426)</sup> Through these effects, there is the potential for antidiabetic therapies to impact on long-term cardiac outcomes in diabetic patients. Sulphonylurea such as glibenclamide, which is used less commonly nowadays, can disrupt cardioprotection through the inhibition of ATP-dependent potassium channels, therefore blocking cardioprotection.<sup>(225, 323, 324)</sup> Glimepiride is able to potentiate and facilitate the ischaemic preconditioning effect.<sup>(395)</sup> Diabetic hearts are resistant to the myocardial infarct limiting effect of IPC.<sup>(395)</sup> Initial findings suggested that this abnormal response of the diabetic heart to IPC might be due to impaired phosphatidylinositol 30-kinase (PI3K)-Akt signalling.<sup>(324)</sup> Yellon's laboratory in 2012 showed that treatment with Glimepiride can overcome this resistance, but the mechanism for this is not clear.<sup>(395)</sup> Metformin is also known to confer cardioprotection in both diabetic and non-diabetic hearts. The cardioprotection mechanism of Metformin is thought to be through inhibition of mitochondrial permeability transition pore opening.<sup>(396)</sup>

In the ERIC- PCI study, Metformin was taken by 19.3% of patients, 20.9% in the control group and (18.6%) in the RIPC group.

## Statins

Administration of statins prior to PCI appears to be effective in reducing myocardial damage during coronary interventions. <sup>(386)</sup> In the experimental settings, statins act as conditioning mimetics. The anti-inflammatory action of statins may influence PCI related events and may reduce PCI-related myocardial injury. <sup>(439)</sup> Although very little is known on the possible interactions of statins with cardioprotection by conditioning strategies, it is now agreed that statins activate the mitochondrial K-ATP channel thereby they could trigger ischaemic preconditioning. <sup>(329)</sup>

Statins increase nitric oxide synthase (NOS) activity and nitric oxide production. <sup>(436)</sup> They also activate the phosphatidylinositol 3-kinase (PI3K)-Akt-endothelial NOS signal transduction pathway. <sup>(436)</sup> These enzymes and kinases are critical parts of conditioning's signal transduction pathway. Of note, Rosuvastatin given orally before elective PCI showed significant improvement in microcirculatory perfusion as assessed by contrast echocardiography. <sup>(440)</sup>

In the ERIC-PCI study, 93% of patients in the control group and 86% of RIPC group were on maintenance dose of statins.

## Beta-Blockers

Beta blockers reduce myocardial oxygen consumption and may have direct cardioprotective effects on the cardiomyocytes. The clinical guidelines recommend beta-blockers as cardioprotective agents in acute coronary syndrome. Beta-blockers have been employed to protect the heart from ischaemia/reperfusion injury in STEMI. The effects of intravenous beta-blockers on myocardial salvage have been investigated in various animal models of acute

ischaemia and reperfusion. The effect of ischaemic preconditioning was abolished in isolated rat hearts after long-term oral treatment with propranolol or nifedipine. <sup>(391,392)</sup>

Zhou et al. in 2013 <sup>(441)</sup> conducted a meta-analysis of 15 randomised-controlled trials in adult cardiac surgery that used RIPC. The authors found that cardioprotection induced by RIPC was less effective and attenuated when beta-blockers were used. In the PPCI setting, a randomised controlled trial by Ibanez et al, <sup>(390)</sup> in 270 patients with anterior Killip class II or less ST-segment-elevation myocardial infarction undergoing PPCI, showed that early intravenous metoprolol before reperfusion reduced infarct size and increased left ventricular ejection fraction during the first 24 hours after STEMI.

## **ACEIs/ARBs**

ACE inhibitors and angiotensin II receptor antagonists when administered before ischaemia and/or reperfusion reduce irreversible myocardial injury, reduce infarct size and interfere with RIPC. <sup>(442)</sup> ACE inhibitors lower the threshold to achieve endogenous cardioprotection, especially in hearts with comorbidities. <sup>(443)</sup>

ACEIs and ARB are not routinely administered prior to elective or primary PCI.

## **Confounding Variables- Nitrate**

As well as treating angina, nitrates are known to have a preconditioning effect. <sup>(279, 393)</sup> and are also licensed for use in cardiac surgery and PCI for their vasodilatory effect. <sup>(444)</sup> Nitrates can induce myocardium protection from ischaemia and reperfusion through a mechanism, which is similar to preconditioning. The issue is that the long term use of nitrates may result in

tolerance and loss of clinical efficacy. This tolerance can aggravate ischaemic/reperfusion injury and decrease preconditioning effect of RIPC. <sup>(426)</sup>

In a human study by Gori et al. (2010) it was reported that the endothelial preconditioning effect of a single dose of nitroglycerin is lost upon a prolonged exposure to nitrate. <sup>(393)</sup> In clinical practice, the findings have been conflicting. The acute administration of nitrates did not appear to interfere with RIPC in patients undergoing coronary artery bypass graft surgery <sup>(445)</sup> but in a recent post-hoc analysis of a RIPC trial on cardiovascular surgery, it was shown that the cardioprotective effect of RIPC was abolished when intravenous glyceryl trinitrate (GTN) was administered intra-operatively, GTN therapy alone actually reduced the extent of perioperative myocardial injury by 39%, suggesting that in itself intraoperative GTN may be cardioprotective. <sup>(446)</sup>

Nitric oxide released by GTN is also known to have several beneficial effects on the cardiovascular system, including protecting the heart against acute ischaemia-reperfusion injury or as a mediator of endogenous cardioprotective strategies such as ischaemic conditioning. <sup>(447)</sup>

Intra-coronary nitrate administered during PCI for alleviation the coronary arteries spasm and accurate estimation of the stent size, was received by 72% of the ERIC-PCI patients without any significant difference between the two groups. Also 40.5% of the patients in the control group and 41.9% of the RIC group were on longterm maintenance treatment with oral nitrate which have had an influence on the outcome of the trial.

## **Confounding Variables- Balloon Inflation within the Stent**

In PCI practice, post-dilation is often performed after stent deployment to improve stent expansion. Occasionally repeated episodes of post- dilation with low-pressure balloon are

required for an optimum result. This is a similar technique used in the postconditioning strategy i.e. conditioning treatment is delivered by periods of balloon inflation and deflation. In the first studies reporting the clinical application of postconditioning in patients with acute MI, it was reported that following stent deployment in the infarct-related coronary artery, interrupting myocardial reperfusion with 4 cycles of one-minute low-pressure inflations and deflations of the coronary angioplasty balloon, myocardial reperfusion was improved. Myocardial infarct size was reduced acutely and at 6 months, the left ventricular function improved at 1 year.<sup>(295, 297, 448)</sup> Although postconditioning is mainly used in acute myocardial infarction for prevention of ischaemic/reperfusion injury, there is also a possibility that this intervention might have an influence on PCI-related myocardial injury.

Although controversial, preconditioning effect has also been observed during coronary occlusions induced by balloon inflation and predilation.<sup>(449)</sup> Some studies have reported that 60 to 120 seconds of occlusion of the coronary artery during the first inflation has been effective in reducing the ST segment level during the second inflation. In 2000, in a well organised trial, preconditioning was observed following 180 seconds of balloon inflation.<sup>(185)</sup>

Although in the ERIC-PCI trial the median pre-dilation and post dilation time is less than 60 seconds, the effect of balloon inflation cannot be entirely ruled out as the optimal duration of inflation to achieve protection is controversial.

In the ERIC-PCI trial, post stent dilatation occurred in 65.8 % of patients in the control group and in 71.1% of patients in the RIPC group. There was no significant difference between the two groups in this regard, P=0.8.

## Confounding Variable- Coronary Collaterals

Coronary Collaterals are present at birth; with wide variation between individuals in their functional capacity. They may develop further in response to obstruction of epicardial coronary arteries to protect jeopardised myocardium to restore blood flow to ischaemic territories. In the course of acute obstruction, a flow of 20% to 25% is sufficient to provide blood supply at rest. However it is generally not sufficient to meet myocardial demands during exercises. The number of collaterals and the extent of their coverage are associated with improved survival in patients with coronary heart disease. In CTO cases with total occlusion, the presence of collaterals supplies blood to the myocardium. This population therefore, can have a low ischaemic burden and the possibility to demonstrate a clear benefit as a result of an anti-infarct intervention is limited. Rentrop is a grading score, designed for assessment of the collateral filling and scores from zero (No collateral filling visible) to grade 3 (complete filling of the epicardial segment of the artery via collateral channels).

Grades of collateral filling	Description
<b>0</b>	None
<b>1</b>	Filling of side branches of the artery via collateral channels without visualisation of the epicardial segment
<b>2</b>	Partial filling of the epicardial segment via collateral channels
<b>3</b>	Complete filling of the epicardial segment of the artery via collateral channels

In the ERIC-PCI study, nearly half of the patients did not have any collaterals and only a minority had grade 3 collateral filling. The differences were not statistically significant.

# **CHAPTER 6**

## **CONCLUSION and FUTURE DIRECTION**

The general consensus amongst almost all clinicians and scientists is that the heart has a remarkable ability to adapt to ischaemic injuries,<sup>(422)</sup> and RIPC of the heart is an adaptive response which has profound cardioprotective effect and enhances the ability of the heart to resist the ischaemic attack. The hallmark of ischaemic conditioning in the laboratory setting, is a reduction of myocardial infarction size, but the outcome of translating this potent cardioprotective effect to the clinical practice in revascularisation settings has been challenging and has been described as conflicting, frustrating and disappointing.<sup>(434)</sup>

Despite extensive laboratory research over the last three decades aiming to achieve cardioprotection via RIPC, and confirmation of proof-of-concept in multiple trials, RIPC remains an experimental technique with limited consistent effect and no clinical application as yet. The inability to fully, consistently, and effectively translate experimentally established reductions in myocardial injury to the bedside is the challenge.

In elective PCI setting, the outcome of the RIPC trials has been variable in terms of reducing the release of cardiac biomarkers, infarct size and MACE.<sup>(15, 9, 209, 211, 214, 217, 416)</sup>

In PPCI setting, the highest risk setting in clinical cardiology, interestingly all trials have reported protection by RIPC, as reflected by reduced release of biomarkers or imaging<sup>(96,208, 210,212, 219, 450)</sup> but currently no guidelines recommend the routine use of this easy and cost free cardioprotective method in clinical practice. Because RIPC appears to be effective and has no known deleterious effect, some interventionalists are already including remote conditioning in their protocol for patients treated with PPCI. The outcome of the adequately powered large trial (ERIC-PPCI) from the Hatter Institute is awaited to confirm whether RIPC will be applicable in clinical practice. The ERIC-PCI trial had a neutral outcome with a possible trend towards positive efficacy of RIPC in reducing Troponin release peri-PCI. The factors contributing to the lack of



desirable achievement in the ERIC-PCI trial are not different from the general pitfalls and obstacles facing other clinical trials in preconditioning, most importantly the small sample size which is the main confounding factor, logistical issues, cardiovascular risk factors and concomitant medication use described in chapter 5.

To achieve consistent cardioprotective results and to ascertain whether RIPC actually improves clinical outcomes in elective PCI, large well-designed and robust randomised controlled trials are required with special attention to the confounding factors. In fact, enhancing our understanding of confounders is a key stepping stone towards clinical translation. <sup>(451)</sup> The potential role of confounding factors needs to be confirmed by designing trials which are designed to recruit these factors. Conversely, the impact of qualitative factors should be minimised to investigate the level of difficulty intrinsic to treating the lesions. Therefore, the development of rational therapeutic approaches to protect the ischaemic heart requires preclinical studies that examine cardioprotection specifically in relation to cardiovascular risk factors and their medications.

As well as recruiting a large number of patients and including confounding factors, trials should also include high-risk patients, who might benefit most from the protection induced by remote ischaemic preconditioning.

It is very important that the 'right' patients are enrolled in the appropriately designed trials and also the 'right' models are chosen in the experimental laboratories to facilitate clinical translation. <sup>(452)</sup> In the experimental laboratories, contrary to the clinical trials where the patient populations are not young, the standard models are young and healthy animals, which do not reflect the fact that cardiovascular disease typically presents in the middle aged and elderly population, therefore do not include the real risk factors and comorbidities such as diabetes. <sup>(422,</sup>

<sup>435)</sup> There is evidence that the cardioprotective effect of preconditioning can diminish with age and diabetes. Furthermore, nitrates, statins, beta-blockers, anti-platelets, ACEs/ARBs, opioids that are common treatments in patients with cardiovascular disease are cardioprotective per se and mimic the benefits of conditioning. Glibenclamide has an opposite effect and prevents conditioning. Based on the data from a trial by Thielmann et al. <sup>(453)</sup> it seems reasonable that future trials should avoid the inclusion of diabetics taking sulphonylureas, but it seems unrealistic to suggest age limitation on recruitment despite preclinical evidence for age-related attenuation of organ protection. <sup>(452)</sup>

In the PPCI setting, patients with small risk regions, extensive collateral perfusion and/or spontaneous reperfusion before PCI which might occur even after passing the guidewire only, will develop small infarcts with subsequent less Troponin release irrespective of treatment. <sup>(452)</sup>

In patients with prolonged ischaemic times, if reperfusion is initiated at about 8 to 12 hours after the onset of symptoms and collateral flow is negligible, evolution of the infarct may be complete, and in the absence of salvageable myocardium, conditioning will again be of negligible benefit. <sup>(452)</sup> These kinds of patients gain minimal benefit from ischaemic conditioning, therefore when designing conditioning trials, specific attention to the role of confounding factors, will enable the trials to provide more robust results with better translation to clinical practice.

In the CABG settings, we need trials that explicitly avoid propofol anaesthesia and concomitant valve surgery and also observe all other established confounders, such as sulfonylureas and possibly nitrates. <sup>(442, 455)</sup>

Regarding the logistics of the preconditioning trials, in most recently published studies, the details and timing of the RIPC algorithm, ischaemic duration, number of balloons inflation and deflation post stent deployment and the method of assessing the infarction size have been

variable. (Table 5, Chapter 5) This variability could indeed be one of the main reasons for the conflicting results; therefore specific attention to the protocols of preconditioning trials is required, aiming to reduce the conflicting outcomes.

Although the ERIC-PCI sample size did not have a robust statistical power to observe confident differences between the treated and the control groups, and included multiple confounding factors that failed to establish the cardioprotection induced by RIPC, the possibility of reaching the ceiling of protection should also be strongly considered. Perhaps the best cardioprotection was already provided when instrumenting the heart, leaving no extra room for additional protective methods. Novel antiplatelet agents, thrombus aspiration, and technical improvements such as soft and flexible wires and catheters, balloons and stents with advanced technology and more importantly the operators' skills, already provided the best cardioprotection and reduced the amount of myocardium at risk during elective PCI, even complex PCI.

This hypothesis is evidenced by the level of 24 hr Troponin (median of 38 ng/Lit) in all recruited patients which is fairly low and non-significant. Of note, only 2 patients in our trial fulfilled the criteria for Type 4a MI diagnosis. The CMR did not show any myocardial oedema or late gadolinium enhancement, even in those two patients who had significant Troponin release.

ERIC-PCI confirms that patients with minimal injuries obtain minimal benefit from ischaemic conditioning. The smaller the injury, the less likely an additional treatment can be effective.

Furthermore, an ongoing controversy exists surrounding the clinical relevance of biomarker-defined peri-procedural myocardial injury. Although it is likely that the ceiling of cardioprotection has been reached in our clinical practice, it must also be highlighted that the

guidelines define peri-PCI MI as elevation of Troponin more than 5 times x 99<sup>th</sup> percentile URL, 48 hour post PCI, plus either (i) evidence of prolonged ischaemia (>20 min) as demonstrated by prolonged chest pain, or (ii) ischaemic ST changes or new pathological Q waves, or (iii) angiographic evidence of a flow limiting complication, such as of loss of patency of a side branch, persistent slow-flow or no-reflow, embolisation, or (iv) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality . This definition is arbitrary chosen and probably with a low threshold. Perhaps a higher level of Troponin post PCI should be defined as the threshold for consideration of PCI-related myocardial injury.

The other debatable hypothesis is that medications routinely prescribed for cardiovascular diseases may show undesirable effects on endogenous cardioprotective cellular signalling mechanisms, by possessing a hidden cardiotoxicity that may manifest latently in the ischaemic heart as increased sensitivity to ischaemic challenge or a decreased capability to adapt to an ischaemic challenge, i.e., attenuated cardioprotection achieved by conditioning.<sup>(451, 454)</sup>

In summary, although the outcomes of the clinical trials in the context of cardiac surgery and elective PCI have been conflicting and translating cardioprotection from the laboratory to the bedside has been challenging, we remain optimistic given the large infarct-sparing effect of RIPC in animal studies and in PPCI setting,<sup>(451, 454)</sup> and look forward to the result of the large randomised controlled trial CONDI2/ERIC-PPCI trial. CONDI investigators in 2016 showed that RIPC as adjunctive to PPCI attenuated the detrimental effect of healthcare system delay on myocardial salvage in patients with STEMI, suggesting that the cardioprotective effect of RIPC increases with the duration of ischaemia.<sup>(456)</sup>

We need trials that are standardised in terms of choosing appropriate patients, design and RIPC protocol, i.e. duration of index ischaemia and location of ischaemic stimulus in upper or lower limb, etc. Trials should reflect a real world situation and take into account as many confounding factor as possible.

In fact there is a critical need to take into account the presence of cardiovascular risk factors and concomitant medications that mimic preconditioning when designing clinical studies. This will hopefully maximise the success rate of developing rational approaches to effective cardioprotective therapies for the majority of patients with multiple risk factors.

## Study Limitations

The major limitation of the ERIC-PCI study was the small sample size. The results might have been different with a statistically significant difference between the two groups, if the study was well powered.

The study design and logistics have probably resulted in inevitable biases. Screening of the eligible patients, recruitment, treatment allocation and randomisation, RIPC intervention, CMR scanning, data collection and outcome analysis were all performed by the main researcher. The SYNTAX scores were calculated entirely by the main researcher's subjective decision. The operating interventionalist was blinded to the treatment allocation but it is likely that the cardiac catheterisation laboratory staff were not all blinded.

Data collection was also not complete due to the logistical difficulties. More importantly 48-hr Troponin concentrations were not tested. CMR scan was abandoned too early following an interim assessment. Haemodynamics, i.e. blood pressure and heart rate was not measured during PCI and retrograde analysis was also not possible.

Although RIPC was applied at an appropriate time before the elective PCI and it was ensured that PCI took place within 1-3 hours of preconditioning to avoid fading of the first protection window, the exact blood pressure cuff to balloon time was not recorded.

Pre infarction angina is associated with cardioprotection and probably represents a clinical correlate of ischaemic preconditioning. Although the patients were advised to avoid exercise on the day before elective PCI, the exact presence or absence of antecedent angina cannot be confirmed.

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