

**THE EFFECT OF REMOTE ISCHAEMIC PRECONDITIONING  
ON CONTRAST INDUCED NEPHROPATHY IN THE CLINICAL  
SETTING OF CORONARY ANGIOGRAPHY AND  
PERCUTANEOUS CORONARY INTERVENTION**

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## DECLARATION

I, Roger Bryan Rear, confirm that the work presented in this thesis is my own. Where information is derived from other sources, I confirm that this has been indicated in the thesis.

**Signed:** \_\_\_\_\_

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## LIST OF ABBREVIATIONS

ACE	angiotensin converting enzyme
ACI	automated contrast injection
ACS	acute coronary syndrome
ADP	adenosine diphosphate
AKI	acute kidney injury
AKIN	acute kidney injury network
AMI	acute myocardial infarction
AMP	adenosine mono-phosphate
ANGII	angiotensin ii
ANT	adeninine nucleotide translocator
ARB	angiotensin receptor blocker
ATN	acute tubular necrosis
ATP	adenosine tri-phosphate
AUC	area under the curve
BD	twice daily
BIVA	bio-impedance vector analysis
CA	coronary angiography
CABG	coronary artery bypass graft
CAD	coronary artery disease
CCF	congestive cardiac failure
CHF	chronic heart failure
CI	confidence interval
CIAKI	contrast induced acute kidney injury
CIN	contrast induced nephropathy
CK	creatinine kinase
CKD	chronic kidney disease
CKD-EPI	chronic kidney disease—epidemiology
CK-MB	creatinine kinase MB
CM	contrast media
CMJ	cortico-medullary junction
COX	cyclooxygenase
CRS	cardio-renal syndrome
CRTP	cardiac resynchronisation therapy pacemaker
CT	computed tomography
CTCA	computed tomography coronary angiography
CTPA	computed tomography pulmonary angiography
CVA	cerebrovascular accident
CVD	cardiovascular disease
DERIC	dual electronic remote conditioning
DM	diabetes mellitus
DNA	deoxyribonucleic acid
DRP1	dynamain-related protein
DVR	descending vasa recta
ECG	electrocardiogram



eGFR	estimated glomerular filtration rate
EPO	erythropoietin
ERK	extracellular signal-regulated kinase
ESRD	end stage renal disease
ESC	European society of cardiology
ESUR	European symposium on urogenital radiology
ET	endothelin
EVAR	endovascular aneurysm repair
FGF	fibroblast growth factor
GC	guanylate cyclase
GCSF	granulocyte-colony stimulating factor
GF	growth factor
GFR	glomerular filtration rate
GLP	glucagon like protein
GMP	guanosine monophosphate
GPCR	g-protein-coupled receptors
GRACE	global registry of acute coronary events
GSK-3 $\beta$	glycogen synthase kinase-3 beta
GTP	guanosine triphosphate
HCT	haematocrit
HD	haemodialysis
HF	haemofiltration
HOCM	high osmolar contrast media
IA	intra-arterial
IABP	intra-arterial balloon pump
IC	ischaemic conditioning
ICAM	intracellular adhesion molecules
ICU	intensive care unit
IGF	insulin like growth factor
IOCM	iso-osmolar contrast media
IPC	ischaemic preconditioning
IPostC	ischaemic post conditioning
IRI	ischaemia reperfusion injury
i.v.	intra-venous
JAK	janus kinase
K/DOQI	kidney disease outcomes quality initiative
KDIGO	kidney disease: improving global outcomes
KIM	kidney injury molecule
LAD	left anterior descending
LDL	low density lipoprotein
LOCM	low-osmolar contrast media
LOS	length of stay
LV	left ventricle
LVEDP	left ventricular end diastolic pressure
LVEF	left ventricular ejection fraction
LVH	left ventricular hypertrophy

MACE	major adverse cardiovascular event
MDRD	modification of diet in renal disease
MI	myocardial infarction
MODS	multi organ dysfunction syndrome
MPTP	mitochondrial permeability transition pore
NAC	n-acetyl cysteine
NADPH	nicotinamide adenine dinucleotide phosphate-oxidase
NGAL	neutrophil gelatinase-associated lipocalin
NHS	national health service
NICE	national institute of clinical excellence
NO	nitric oxide
NOS	nitric oxide synthase
NYHA	New York health association
OR	odds ratio
PAF	platelet activating factor
PCI	percutaneous coronary intervention
PI3K	phosphoinositide 3-kinase
PKC	protein kinase c
PKG	protein kinase g
PPCI	primary percutaneous coronary intervention
PT	proximal tubular
PTCA	percutaneous transluminal coronary angioplasty
PTEN	phosphatase and tensin homologue
RCT	randomised controlled trial
RIC	remote ischaemic conditioning
RIFLE	risk, injury, failure, loss, end stage
RIPC	remote ischaemic preconditioning
RIPostC	remote ischaemic post-conditioning
RISK	reperfusion injury salvage kinase
ROS	reactive oxygen species
RPCT	remote ischemic preconditioning of trauma
RR	relative risk
RRT	renal replacement therapy
SAFE	survival activating factor enhancement
SCr	serum creatinine
SIRS	systemic inflammatory response syndrome
STAT	signal transducer and activator of transcription
STEMI	ST elevation myocardial infarction
SWOP	second window of protection
TAVI	transcatheter aortic valve implantation
TGF	tubulo-glomerular feedback
TNF- $\alpha$	tumour necrosis factor alpha
UACR	urine albumin creatinine ratio
UCLH	University College London Hospital
UK	United Kingdom
WRF	worsening of renal function

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# CHAPTER 1: INTRODUCTION

## 1.1 CARDIOVASCULAR AND KIDNEY DISEASE

Cardiovascular disease (CVD) encompasses a wide range of acute and chronic pathologies, specifically atherosclerotic, myocardial, valvular and pericardial disease as well as cardiac arrhythmias. Kidney disease likewise incorporates a myriad of vascular, glomerular, tubulo-interstitial and obstructive nephropathies, presenting either abruptly as an acute kidney injury (AKI) or progressing insidiously in chronic kidney disease (CKD). Co-existence of cardiovascular and kidney disease is a frequent clinical finding, determined by shared aetiologies and numerous bidirectional pathological interactions, which confers additional risk and which carries an ominous prognosis.

The traditional risk factors associated with cardiovascular disease, namely advanced age, male gender, Asian ethnicity, diabetes mellitus, hypertension, dyslipidaemia as well as lifestyle factors including smoking, physical inactivity and obesity, overlap considerably with the risk factors for CKD (Table 1). In studies of patients with established CKD, up to 20% have concurrent diabetes mellitus and 50% suffer from hypertension<sup>1</sup>. Similarly in patients with established diabetes or hypertension, the prevalence of CKD has been found to be as high as 26% and 37% respectively<sup>2</sup>. Throughout the world these risk factors are now epidemic, with one in seven people treated for hypertension<sup>3</sup> and one in twelve people diagnosed with diabetes<sup>4</sup>. These totals are projected to increase by 50% over the next decade and the prevalence of CKD is expected to rise accordingly.

As would be expected by way of shared aetiology alone, notwithstanding the independently deleterious effect that CKD has on the cardiovascular system, CVD is highly prevalent in renal patients and is directly proportional to the severity of renal dysfunction. One in three patients with moderate to severe CKD will suffer from coronary artery disease (CAD) and a third develop chronic heart failure (CHF).<sup>1</sup>

Similarly a considerable proportion of patients with CVD have co-existent CKD, found in a third of patients undergoing coronary angiography<sup>5-7</sup> and in a third with chronic heart failure. Co-existent CVD and CKD leads to exacerbation of disease progression in both conditions and considerably increases morbidity and mortality. A systematic review by Tonelli et al<sup>8</sup>, including 100,064 patients with mild to moderate CKD, found that at age 50 years, the relative risk of cardiovascular mortality was 3.4 (95% CI 2.1 to 5.5) whereas at 70 years, the relative risk was 1.5 (95% CI 0.96 to 2.3). By the onset of end stage renal disease (ESRD) and dialysis, even after stratification for age, gender, race and diabetes, cardiovascular mortality is 10 to 20 times higher than that of the general population<sup>9</sup>.

Pre-existing CKD or the onset of AKI significantly increases the risk of serious adverse events following cardiovascular investigations and therapies. A prototypical example of this is ‘contrast induced nephropathy’ (CIN) which describes an acute kidney injury caused by exposure to the intravascular radio-contrast media administered during coronary angiography (CA) and which is associated with significant major adverse cardiac events (MACE) . Clinical concerns regarding the increased risks brought about by CIN following CA based cardiac investigations may lead to delay or even withdrawal of prognostic therapies which must be carefully balanced against the potential benefits in this high risk cohort. In light of these concerns, patients with both CVD and CKD are optimally managed by multidisciplinary teams with experience of the complexities within this clinical field.

**Table 1: Common risk factors for CVD and CKD**

<b>CKD specific risks</b>	<b>Shared CVD and CKD risks</b>	<b>CVD specific risks</b>
Autoimmune disease	Age	Genetic disease
Vasculitic disease	Male sex	Infectious disease
Cirrhotic liver disease	Black/Asian ethnicity	Pulmonary disease
Polycystic Kidney Disease	Hypertension	Thrombophilia
Pyelonephritis	Diabetes	Chemotherapy
Glomerulonephritis	Dyslipidaemia	Radiotherapy
Nephrotoxic agents	Smoking	Physical inactivity
Urinary outflow obstruction	Obesity	Dietary factors

### 1.1.1 ACUTE KIDNEY INJURY AND CHRONIC KIDNEY DISEASE

The manifold aetiologies of both AKI and CKD can be broadly categorised into pre-renal, renal and post-renal processes.

Pre-renal insults involve disruption of renal perfusion due to a reduction in circulating blood volume (e.g. haemorrhage or over-diuresis), reduction in cardiac output (e.g. cardiogenic shock or low output cardiac failure), renal arterial compromise (e.g. renal artery stenosis or excessive vasoconstriction) or venous congestion (e.g. venous thrombosis or congestive heart failure).

Renal (or intrinsic) aetiologies are caused by primary parenchymal disease, which can be subdivided by the structures principally affected, notably the glomerulus, tubules or interstitium as well as those secondary to systemic disease processes (e.g. diabetes mellitus and autoimmune disease) or specific to the kidney (e.g. polycystic kidney disease or pyelonephritis).

Post renal pathologies involve obstruction of urinary outflow from the kidney either at a tubular (e.g. crystal deposition) or anatomical level (e.g. prostatic hypertrophy). Severe AKI in which permanent renal injury occurs may herald the onset of CKD, with loss of renal auto-regulatory processes and development of systemic complications secondary to kidney dysfunction (e.g. hypertension) leading to ongoing renal injury and a progressive decline in glomerular filtration rate (GFR) (Table 2).

Following an acute renal insult, the diagnosis of AKI typically relies upon an initial reduction in urinary output lasting at least 6 to 12 hours followed by a rise in serum creatinine (SCr), or a fall in estimated glomerular filtration rate (eGFR - calculated using SCr and adjusted for variables such as age, gender, ethnicity and weight). Elevations in SCr are maximal after 48 hours and may persist for one to two weeks before gradually falling, although if significant injury has occurred SCr levels may never return to baseline. A number of graded classification systems for AKI exist as summarised in Table 3.

The diagnosis of CKD is dependent on a persistent reduction in eGFR for a minimum of three months, to under 60ml/min/1.73m<sup>2</sup>, or under 90ml/min/1.73m<sup>2</sup> in the presence of structural kidney abnormality, albuminuria, or genetic disease. The Kidney Disease Outcome Quality Initiative (K/DOQI), classification of CKD consists of five categories that correspond with the severity of disease, therapeutic modalities and prognosis (Table 4)<sup>10</sup>.



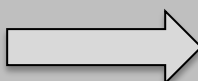
### 1.1.1.1 GFR, serum creatinine and estimated GFR measurement

Currently all standard clinical investigations for assessment of renal function have limitations and do not correlate well with true GFR, defined as the volume of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit of time. Differential basal tone of the afferent and efferent glomerular arterioles provides homeostasis of GFR, regulated by neuronal and hormonal mediators. GFR is equal to the 'Clearance Rate' of a solute, if freely filtered and neither reabsorbed nor secreted by the kidney. Serum creatinine (SCr), formed by the breakdown of creatine phosphate in muscle tissue, is freely filtered by the glomerulus but also actively secreted in small amounts by the peritubular capillaries, leading to an overestimation of GFR of between 10% and 20%. SCr measurement is a widely available investigation, limited by dependence on a number of non-renal factors such as total body pool of creatine phosphate (total muscle mass), creatine phosphate generation rate, conversion rate of creatine phosphate to creatinine, dietary sources of creatinine, hydration status, renal tubular secretion rate of creatinine, urinary flow rate and assay interference. As such different SCr concentrations can occur between individuals with the same renal function due to differing age, gender and ethnicity and other factors. Several calculations have been developed to provide a more accurate estimation of true GFR by incorporating known clinical variables, for example that of the Modified Diet in Renal Disease (MDRD) study group<sup>11</sup>.

$$\mathbf{eGFR\ MDRD = 186 \times (SCr / 88.4)^{-1.154} \times (Age)^{-0.203} \times (0.742\ \text{if female}) \times (1.210\ \text{if black})}$$

Criticisms of this formula include the absence of total body weight, as in the original Cockcroft-Gault formula<sup>12</sup>, as well as reduced accuracy in patients with GFR >60ml/min. As such adoption of the more accurate CKD-EPI formula<sup>13</sup> is now commonplace, which has also been shown to correlate more precisely with cardiovascular endpoints<sup>14</sup>.

**Table 2: Aetiology of AKI and CKD**

<b>Acute Kidney Injury</b>  <b>Chronic Kidney Disease</b>	
<b>Pre- Renal</b>	Acute hypo-perfusion <ul style="list-style-type: none"> <li>• Haemorrhage/dehydration</li> <li>• Sepsis/cardiogenic shock</li> </ul> Vascular <ul style="list-style-type: none"> <li>• Embolism</li> <li>• Vasoconstriction (drugs/<math>Ca^{2+}</math>)</li> <li>• Renal vein thrombosis</li> </ul>
<b>Renal</b>	Acute Glomerulonephritis <ul style="list-style-type: none"> <li>• IgA/haemolytic uremic syndrome</li> </ul> Acute Tubular Necrosis <ul style="list-style-type: none"> <li>• Contrast induced nephropathy</li> <li>• Nephrotoxic</li> </ul> Acute Interstitial Nephritis <ul style="list-style-type: none"> <li>• Allergic</li> </ul>
<b>Post- Renal</b>	Chronic Urinary Retention <ul style="list-style-type: none"> <li>• Benign prostatic hypertrophy</li> <li>• Neuropathic bladder</li> <li>• Urolithiasis/malignancy</li> </ul> Tubular Obstruction <ul style="list-style-type: none"> <li>• Crystal/Protein</li> </ul> Vesico-ureteric reflux

**Table 3: Three classification systems for severity of AKI**

RIFLE <sup>15</sup>		AKIN <sup>16</sup>		KDIGO <sup>17</sup>		All
Stage	SCr/eGFR (7 days)		SCr (48 hr)		SCr (48hr)	Urine output
<b>Risk</b>	SCr x 1.5 eGFR < 25%	<b>1</b>	SCr x 1.5-2 or >0.3mg/dl	<b>1</b>	SCr x 1.5 -1.9 or >0.3mg/dl	<0.5ml/kg/hr for 6hr
<b>Injury</b>	SCr x 2 eGFR <50%	<b>2</b>	SCR x 2-3	<b>2</b>	SCr x 2-2.9	<0.5ml/kg/hr for 12 hr
<b>Failure</b>	SCr x 3 (or >0.5mg/dl if baseline >4mg/dl) eGFR <75%	<b>3</b>	SCr x 3> or >0.5mg/dl if baseline >4mg/dl) or RRT	<b>3</b>	SCr x 3> or >4mg/dl or RRT	<0.3ml/kg/hr for 24 hr or anuria for 12hr
<b>Loss</b>	AKI >4 weeks					
<b>ESRD</b>	ESRD >3 months					

SCr – Serum Creatinine, eGFR = estimated glomerular filtration rate, RRT = Renal replacement therapy, AKI = Acute kidney injury, ESRD = End stage renal disease, hr = hour

**Table 4: The K/DOQI Chronic kidney disease classification system**

K/DOQI <sup>10</sup> Stage	GFR ml/min	Description	Treatment
<b>I</b>	>90	Normal kidney function with urinary, structural or genetic abnormality	Observation, blood pressure and cardiovascular risk management
<b>II</b>	60-89	Mild reduction in kidney function	As above
<b>IIIa</b>	45-59	Moderate reduction in kidney function	As above
<b>IIIb</b>	30-44		
<b>IV</b>	15-29	Severe reduction in kidney function	As above, planning for end stage kidney failure,
<b>V</b>	<15 Dialysis	Very severe or 'end stage' renal disease	Haemodialysis, Renal transplant, anaemia, calcium, cardiovascular risk management
<b>P/T/D</b>	P = Proteinuria, T= Renal Transplant, D= Dialysis		

### 1.1.2 CORONARY ARTERY DISEASE

Coronary artery disease (CAD), otherwise known as ischaemic heart disease (IHD) or coronary heart disease (CHD) encompasses a spectrum of clinical presentations including stable angina, the acute coronary syndromes (ACS) namely unstable angina (UA), non-ST elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI) as well as sudden cardiac death (SCD). CAD is characterised by atherosclerosis of one or more of the coronary arteries, which may limit coronary blood flow causing myocardial ischaemia, or lead to myocardial infarction following atherosclerotic plaque rupture and thrombotic occlusion of the vascular lumen.

CAD remains the leading cause of mortality in the world, responsible for almost 30% of all deaths<sup>18</sup>. In the last decade in the United Kingdom, cancer has overtaken myocardial infarction as the leading cause of death in men, however it remains the primary cause of death in women<sup>19</sup>. The rising global incidence of CAD risk factors and an aging population will ensure that CAD remains the major future challenge facing health care systems in both the developed and developing world.

### 1.1.3 CORONARY ARTERY DISEASE AND CHRONIC KIDNEY DISEASE

Accelerated atherosclerosis of the coronary arteries, and indeed of any artery, is pathognomic of the interaction between CVD and CKD. Beginning in Stage I CKD, low-grade micro-albuminuria, defined as a urine albumin creatinine ratio (UACR) of  $\geq 2.5$  mg/mmol in men and  $\geq 3.5$  mg/mmol in women, is associated with an increased risk of adverse cardiovascular outcomes, even in non-diabetic, non-hypertensive patients and is now recognised as an independent cardiovascular risk factor<sup>20</sup>. As eGFR declines, CAD risk has been shown to increase proportionately<sup>21</sup> with a twofold increase in the rate of CAD progression<sup>22</sup> and an increased risk of death following MI in patients with moderate to severe CKD<sup>23</sup>. In patients with established renal disease, cardiovascular mortality is 10-30 times higher,<sup>1</sup> and is a more likely to occur than progression to end-stage renal disease (ESRD)<sup>24</sup>.

The rapid progression of CAD seen in CKD is a direct result of the complex pathophysiology inherent in renal dysfunction with promotion of vascular calcification<sup>25</sup> and endothelial dysfunction secondary to factors such as renin-angiotensin dysregulation, hyper-uricaemia, hyper-parathyroidism and hyper-phosphataemia<sup>26</sup>, increased oxidative stress, systemic inflammation<sup>27</sup>, and hyper-homocysteinaemia<sup>28</sup>. In addition physiological stressors such as hypertension, hypervolaemia, malnutrition and anaemia<sup>29</sup> as well as pro-thrombotic states<sup>30</sup> are highly contributory elements. As with all preventive strategies employed against CAD, a multidisciplinary approach to control the progression of both renal and cardiovascular disease is essential to reduce the excessively high morbidity and mortality rate in this patient cohort.<sup>31</sup>

#### 1.1.4 THE 'CARDIO-RENAL' SYNDROME

The complex bidirectional pathophysiological interaction between the heart and kidney has been termed the 'cardio-renal syndrome'<sup>32</sup> (CRS), which is further classified based upon primary organ dysfunction and chronicity (see table 5). In primary cardiac dysfunction, an acute (type 1) or chronic (type 2) reduction in cardiac output compromises renal arterial blood flow, whilst venous congestion, commonly seen in both systolic and diastolic heart failure, reduces the renal perfusion gradient and increases the risk of renal ischaemia<sup>33</sup>.

In primary renal disease an acute (type 3) or chronic (type 4) reduction in renal function leads to neuro-hormonal dysregulation, electrolyte and calcium disturbances, anaemia, hypertension, volume overload, oxidative stress and activation of inflammatory cascades. This in turn has a deleterious effect on cardiac function, in both an acute (e.g. decompensated heart failure, myocardial infarction and arrhythmia) and a chronic fashion (e.g. accelerated coronary artery disease (CAD), left ventricular hypertrophy (LVH), and vascular calcification). Type 5 CRS occurs when both organ systems are simultaneously affected by severe systemic disease processes such as sepsis and toxemia. It is important to recognise that following the onset of CRS, a negative feedback loop may exist where dysfunction of the secondary organ further exacerbates dysfunction of the primary organ.

Table 5: The ‘cardio-renal’ syndrome, adapted from the 2010 ADQI conference<sup>32</sup>

	<b>Inciting event</b>	<b>Secondary disturbance</b>	<b>Example</b>
<b><u>Type 1</u> Acute cardio-renal syndrome</b>	Acute cardiac dysfunction	Acute kidney injury	Acute cardiogenic shock or acute decompensation of chronic heart failure
<b><u>Type 2</u> Chronic cardio-renal syndrome</b>	Chronic cardiac dysfunction	Chronic kidney disease	Chronic heart failure
<b><u>Type 3</u> Acute reno-cardiac syndrome</b>	Acute kidney injury	Acute cardiac dysfunction (e.g. heart failure or arrhythmia)	Contrast induced nephropathy
<b><u>Type 4</u> Chronic reno-cardiac syndrome</b>	Chronic kidney disease	Chronic cardiac dysfunction and/or increased risk of MACE	Diabetic renal disease
<b><u>Type 5</u> Secondary cardio-renal syndrome</b>	Systemic disease	Cardiac and renal dysfunction	Systemic lupus, sepsis

## 1.2 CORONARY ANGIOGRAPHY & PERCUTANEOUS CORONARY INTERVENTION

Invasive coronary angiography was pioneered in 1958 in the United States by Sones and Proudfit<sup>34</sup> and remains the gold standard for coronary artery imaging. Under local anaesthesia an arterial sheath is sited into either the femoral, brachial or radial artery to allow introduction of the cardiac catheter. This is a long, flexible, specifically shaped radio-opaque tube, which is guided into the ascending aorta and engaged into the ostium of either the right or left coronary artery under x-ray video fluoroscopy. Once engaged, radio-opaque contrast media (CM) is injected into the coronary artery to directly visualise the coronary anatomy. CM may also be injected into the left ventricle to assess myocardial and valvular function (left ventriculography) and into the aorta to visualise aortic anatomy (aortography). Haemodynamic pressure measurements within the aorta and left ventricle as well as continuous ECG recordings are acquired during the procedure in order to assess patient stability and allow additional cardiovascular diagnosis.

Percutaneous coronary intervention (PCI), otherwise known as percutaneous transluminal coronary angioplasty (PTCA) was developed by Andreas Gruntzig<sup>35</sup> in 1977 as a minimally invasive intervention to treat coronary artery stenoses in order to restore normal coronary blood flow. Following on from coronary angiography, a guide catheter and guide wire is used to position a balloon tipped catheter within the 'target' coronary stenosis, which is then inflated to restore patency of the arterial lumen. This technique has since been modified to allow deployment of metallic stents directly within coronary stenoses in order to stabilise atherosclerotic plaque and to structurally support the lumen of the vessel; this development has been shown to be superior to balloon angioplasty alone<sup>36</sup> and is now the treatment of choice unless stent insertion is not possible (e.g. contra-indication to dual antiplatelet therapy required for maintenance of stent patency).



### **1.2.1 COMPLICATIONS OF CORONARY ANGIOGRAPHY AND PCI**

Diagnostic CA carries a relatively low risk of adverse events, although this is highly dependent on patient factors such as advanced age, left ventricular dysfunction, hypotension, peripheral vascular disease and renal dysfunction. In general CA involves an approximate 2% risk of major complications including MI, cerebrovascular accident (CVA), emergency CABG, ventricular arrhythmia, major haemorrhage or vascular injury and a 0.08% risk of death<sup>37</sup>. PCI carries a greater risk, related to patient specific factors in the same way as CA, but also related to procedural factors such as the urgency of the procedure and complexity of the target coronary lesions<sup>38</sup>.

### **1.2.2 COMPLICATIONS OF CONTRAST MEDIA ADMINISTRATION**

Approximately 0.2% of patients receiving modern non-ionic CM may experience a Type 1 (Mast Cell/Histamine/IgE mediated) allergic reaction, particularly if a prior history of asthma or atopy, allergy to CM or Iodine exists<sup>39</sup>. This can precipitate an urticarial rash, angioedema, acute asthma, allergic vasculitis and rarely anaphylaxis (0.02%). This is usually prevented or treated with antihistamines and steroid medications<sup>40</sup> and if severe allergy occurs, airway management,  $\beta$ -agonist bronchodilators, intravenous crystalloids and epinephrine may be required.

In susceptible individuals, intravascular administration of highly viscous and often hyperosmolar radio-contrast media may lead to an acute kidney injury (AKI) known as contrast induced nephropathy (CIN), which will be examined comprehensively in the following chapter. Because CIN provides a predictable model of renal ischaemia reperfusion injury (IRI) and remains a significant iatrogenic clinical problem, it is an attractive target for basic and clinical research into novel prophylactic interventions.

### 1.3 CONTRAST INDUCED NEPHROPATHY

Contrast induced nephropathy (CIN), also commonly referred to as contrast induced acute kidney injury (CIAKI), is an iatrogenic kidney injury that follows intravascular administration of radiopaque CM in at risk patients. The first cases of CIN were identified in the 1950's, after intravenous pyelography was performed in patients with multiple myeloma associated renal disease, leading to acute renal failure and death<sup>41,42</sup>. Even today, CIN continues to be responsible for a third of all cases of hospital acquired AKI<sup>43,44</sup> affecting up to 1-2% of all patients and occurring in up to 50% of higher risk patients undergoing CA or PCI<sup>45</sup>.

At present in Europe approximately one third of patients undergoing CA or PCI will have stage III-V CKD which is recognised as the main risk factor for CIN. The mean rate of CIN in at risk patients undergoing CA or PCI using optimal prophylactic measures is between 10% and 15%<sup>46</sup>. In the UK approximately 400,000 people undergo CA or PCI<sup>47</sup> per year, equating to 120,000 patients at risk of CIN, of which 12-18,000 are estimated to develop CIN. In most cases of CIN, renal function will completely recover however in an estimated 2400 to 3600 cases an irreversible deterioration in renal function will occur. A small number of these patients will, as a result, require long-term renal dialysis which is a serious adverse outcome and carries significant costs for the NHS. Taking into consideration the cost of extra bed days alone, the average length of stay (LOS) for a patient who undergoes CA or PCI and develops CIN is  $6.8 \pm 7.1$  days vs.  $2.3 \pm 2.5$  days for patients without CIN<sup>48</sup>. Assuming the cost of a bed day in the UK is £225<sup>27</sup>, the estimated index hospital costs due to CIN, not including dialysis, readmissions, or mortality is £1012 per CIN patient; which constitutes a total cost to the NHS of approximately £12.1-18.2 million per year.

A wide number of modern cardiac imaging and interventional procedures require administration of intravascular CM, including CT coronary angiography (CTCA), transcatheter aortic valve Implantation (TAVI) as well as cardiac resynchronisation therapy pacemaker (CRTP) implantation. Primary PCI, developed for the emergency treatment of acute myocardial infarction (AMI), has revolutionised cardiovascular outcomes, albeit at the expense of increased rates of CIN. This is due to the presence of unknown pre-procedural CIN risk factors, such as reduction in eGFR, as well as known risk factors such as the time dependent inability to provide adequate i.v. pre-hydration, peri-procedural hypotension and larger volumes of CM required<sup>49</sup>.

CM is also commonly used in many non-cardiac imaging modalities such as CT pulmonary angiography (CTPA) and indeed any plain or CT imaging of a vascular bed (e.g. femoral angiography, CT cerebral angiography etc.) or of the urological system (e.g. intravenous urography). In addition to the greatly expanded number of imaging modalities requiring CM, the population undergoing CM based investigations are at an inherently greater risk of CIN due to the greater incidence of patients with advanced age and co-morbidity, with the result that CIN remains a growing and significant clinical problem<sup>50</sup>.

Patient specific risk for the development of CIN can be estimated using known demographic, clinical and peri-procedural factors. Although the classical risk factor for CIN is the presence of chronic kidney disease (CKD) stage III or worse<sup>10</sup> (Table 4), numerous additional risk factors are also highly contributory to CIN<sup>45</sup>, many of which are perhaps less well recognised in clinical practice. Cohort studies investigating CIN<sup>51, 52</sup> have led to the development of several risk scoring systems, enabling early prediction of CIN and prompting provision of additional prophylactic measures. The predictable and serious nature of CIN continues to encourage a wealth of basic and clinical research into this common iatrogenic complication.

### 1.3.1 DEFINITION AND DIAGNOSIS OF CIN

A relative 25% increase in serum creatinine (SCr), or an absolute increase of 0.5 mg/dl (44µmol/L) from baseline SCr, within 72 hours of contrast exposure without alternative explanation, is the most commonly used definition of CIN<sup>53</sup>. This definition has been criticised as even minor increases in SCr have been shown to correlate with significant renal injury and adverse events<sup>54,55</sup> and no reference is made to early functional reductions in urine output, such as in the RIFLE<sup>15</sup>, AKIN<sup>16</sup> and KDIGO<sup>17</sup> classification systems (Table 4). However this definition is easy to implement in clinical practice and has been widely adopted as the primary endpoint in most CIN studies and as such has been convincingly associated with adverse clinical outcomes. Harjai et al<sup>56</sup> have further refined the classical CIN definition using three grades of relative and absolute creatinine increase, including minor changes in SCr (<25% or 0.5mg/dl), which have also been shown to correlate with adverse clinical outcomes (Table 6).

**Table 6: CIN Grades, adapted from Harjai et al<sup>56</sup>**

CIN Grade	Change in Serum Creatinine	6 month Outcomes
<b>Grade 0</b>	SCr increase <25% and <0.5 mg/dL above baseline	MACE 12.4% Mortality 10.2%
<b>Grade 1</b>	SCr increase >= 25% and <0.5 mg/dL above baseline	MACE 19.4% Mortality 10.4%
<b>Grade 2</b>	SCr increase >= 0.5 mg/dL above baseline	MACE 28.6% Mortality 40.9%

CIN = Contrast Induced Nephropathy, SCr = Serum Creatinine, MACE = Major Adverse Cardiovascular Events

### **1.3.2 NOVEL BIOMARKERS FOR THE DETECTION OF CIN**

The clinical utility of SCr is limited by the delayed response to the rapid changes in GFR that occur in AKI, particularly in patients with normal preceding renal function,<sup>57</sup> with increases in SCr typically requiring 48 hours to breach a diagnostic threshold. Additionally renal function must decrease by more than half before a significant increase in SCr is observed, limiting its sensitivity considerably. Numerous renal biomarkers have been developed that aim to provide earlier and more sensitive diagnosis, including neutrophil gelatinase associated lipocalin (NGAL)<sup>58</sup>, urine albumin creatinine ratio (UACR), cystatin c<sup>59</sup>, urinary Kim-1<sup>60</sup>, IL-18<sup>61</sup> and L-FABP<sup>62</sup>. Although promising results have been demonstrated in small clinical trials, these novel biomarkers have yet to be translated into clinical practice and they await validation in the early diagnosis of AKI and as predictors of adverse clinical outcomes in larger studies.

#### **1.3.2.1 Neutrophil gelatinase associated lipocalin (NGAL)**

NGAL is a small (25 kDa) protein secreted by many organs (including the kidney) in response to ischaemia, whose role is to scavenge free extracellular iron released from injured tissues. Liberated iron is a noxious substance and is responsible for catalysing hydrogen peroxide into hydroxyl anion, a reactive oxygen species (ROS) involved in ongoing cellular injury. McCullough et al demonstrated that NGAL is a sensitive, although somewhat less specific biomarker useful in the early detection of CIN<sup>63</sup>, especially useful in patients who develop a subclinical kidney injury, as defined by a SCr rise of 0-25% above baseline. Acute elevation in NGAL is detectable in both serum and urine samples within a few hours of renal injury that makes this biomarker a promising tool for the early detection of AKI. Point of care (POC) testing equipment is available to further reduce the diagnostic lead time, enabling prompt risk stratification and therapeutic interventions.

### **1.3.2.2 Urine Albumin Creatinine Ratio (UACR)**

Spot UACR, a widely available and cost effective investigation, measures the ratio of urine albumin to urine creatinine at a given time point. This measure takes into account variations in urinary concentration and is a surrogate for 24-hour urine albumin excretion, albeit assuming constant serum creatinine concentration. Micro-albuminuria, defined as an UACR  $\geq 3.5$  mg/mmol (female) or  $\geq 2.5$  mg/mmol (male), can be a manifestation of endothelial dysfunction resulting from hypertension and diabetes and is proportional to the severity of kidney disease and strongly correlated with CVD and mortality<sup>64</sup>. Microalbuminuria is a recognized risk factor for AKI as well as for progression of CKD, mediated by mechanisms including inhibition of podocyte regeneration at the glomerular tuft<sup>65</sup>. Development of new or worsening microalbuminuria secondary to acute glomerular injury leads to proximal tubular toxicity and dysfunction, mediated by reactive oxygen species (ROS) and protein kinase C (PKC)<sup>66</sup>. UACR has been shown in clinical trials to predict the progression and severity of AKI following cardiac surgery<sup>67</sup> and has been suggested as a sensitive marker for CIN at a threshold below the sensitivity of observed changes in SCr or eGFR<sup>68</sup>.

### **1.3.2.3 Cystatin C**

Cystatin C is a small (13 kDa), non-glycosylated basic protein belonging to the cysteine protease inhibitor family and is produced by all nucleated cells. It is a useful marker of renal function as it is freely filtered at the glomerulus, almost completely reabsorbed and is not secreted by the proximal renal tubular cell; as such the plasma concentration of Cystatin C is almost exclusively determined by the GFR. In addition it is not affected by muscle bulk or age which complicate the interpretation of SCr. Briguori et al<sup>59</sup> demonstrated that Cystatin C elevation of  $>10\%$  from baseline was a more sensitive test than SCr, with diagnosis of CIN possible at 24 hours following CM exposure rather than at 48 hours as with SCr. In addition Cystatin C elevation also correlated well with major adverse events at one year.

#### **1.3.2.4 Urinary Kidney Injury Molecule 1 (KIM-1)**

KIM-1 is a transmembrane protein upregulated in proximal tubular cells following ischaemic injury and is subsequently shed into the urine. It has been shown to be specific for ATN over other forms of renal injury based upon renal biopsy analysis<sup>69</sup>. Torregrosa et al recently demonstrated that urinary KIM-1 elevation at 12hours is a useful predictor of AKI following coronary angiography although at a lower sensitivity and specificity than NGAL<sup>70</sup>.

#### **1.3.2.5 Interleukin 18 (IL-18)**

Interleukin 18 is a pro-inflammatory cytokine produced by macrophages and other leukocytes in response to a wide range of triggers, including ischaemia, and is involved in activation of the cell mediated immunity response. Variable predictive accuracy has been reported with urinary IL-18 as a marker of AKI, however a well conducted recent meta-analysis by Liu et al suggested that urinary IL-18 is a useful predictor of AKI and is consistent with SCr measurement in a broad cohort of patients, including those post CA/PCI, post CABG and on intensive care units<sup>71</sup>

#### **1.3.2.6 Liver type fatty acid binding protein (L-FABP)**

Urinary excretion of liver type fatty acid binding protein (L-FABP) is a biomarker of tubulo-interstitial damage arising from ischaemic and toxic renal injury, with peak levels rising within 24 hours after contrast administration, enabling early detection of CIN. Igarashi et al<sup>62</sup> demonstrated that RIPC attenuated urinary L-FABP after CA in patients at low to moderate risk of CIN, albeit without significant differences in SCr between control and RIPC groups. Correlation with adverse clinical outcomes following CIN is yet to be established.

### 1.3.3 ADVERSE OUTCOMES FOLLOWING CIN

Although CIN is often considered a transient event, with up to 80% of patients recovering renal function within one to three weeks<sup>72</sup>, observational clinical studies have demonstrated that it is associated with adverse clinical outcomes over the short and long term<sup>73</sup>. In-hospital mortality after CIN has been shown to be up to five times greater, even after adjusting for co-morbidities<sup>74</sup> and longer term mortality risk at one and five years has been found to be four times higher<sup>75</sup>, with some studies revealing a one year mortality risk of between 20%<sup>55</sup> and 38%<sup>76</sup>. Of those patients who develop CIN, up to 20% suffer persistent worsening renal function (WRF)<sup>5</sup>, with acute renal replacement therapy becoming necessary in 0.7%<sup>74</sup> to 7%<sup>76</sup> of patients (Table 7).

Not surprisingly, the additional healthcare costs incurred due to CIN are considerable<sup>55</sup>. However drawing conclusions from these observational studies is problematic as they cannot establish a direct causal relationship between CIN and mortality. For example, a severe cardiac insult, which itself carries a poor prognosis, is likely to be associated with an increased risk of CIN and thus CIN may only function as a marker of adverse events. James et al<sup>77</sup> performed a meta-analysis on 39 observational studies investigating CIN and demonstrated increased mortality, cardiovascular events, persistent WRF and prolonged hospital stay, although strong confounders were present in the clinical characteristics that predispose to both CIN and mortality. Even after appropriate adjustment for these confounders, the authors advise caution in making any firm conclusions regarding causality.



There are several conceivable pathological mechanisms that might explain causality between CIN and major adverse cardiac events (MACE). During the acute phase of CIN, acute volume overload, uraemia, electrolyte imbalance and the pro-inflammatory and pro-thrombotic milieu associated with AKI may result in an acute cardiac insult, otherwise described as type 3 cardio-renal syndrome. In addition there is an added risk of complications arising from invasive therapies such as haemofiltration or dialysis. Those patients that suffer persistent worsening of renal function may develop type 4 cardio-renal syndrome due to progressive atherosclerosis, vascular calcification and left ventricular hypertrophy<sup>21</sup>. However in the majority of patients with only minimal or transient changes in renal function it remains speculative how CIN might lead to increased MACE.

If well designed interventional studies are able to demonstrate that effective CIN prevention strategies reduce MACE, without using therapies that might provide an independent cardiovascular benefit, the question of causality between CIN and MACE may finally be answered. An alternative approach, which side steps some of the confounders that exist between CIN and MACE, is for clinical trials to concentrate on adverse renal outcomes, such as persistent WRF, proteinuria and progression to ESRD. Regardless of direct causality, CIN is a worrying clinical occurrence that should when and wherever possible be pre-empted with appropriate prophylactic measures.

**Table 7: Cardiovascular adverse outcomes following CIN**

Adverse Event	Outcome CIN vs no CIN
In-Hospital Mortality	7.1% vs 1.1% (P<0.0000001) McCulloch et al <sup>74</sup> , N=1826
1 Year Mortality	37.7% vs 19.4% (P=0.001) Gruberg et al <sup>76</sup> , N= 439
Persistent Worsening Renal Function (eGFR>25% baseline at 3/12)	18.6% vs 0.9% (P=0.0001) Maoili et al <sup>5</sup> , N=1490
Haemodialysis	0.7% McCulloch et al <sup>74</sup> 7% Gruberg et al <sup>76</sup>

CM = Contrast media exposure, CIN = Contrast Induced Nephropathy, eGFR= Estimated glomerular filtration Rate

### 1.3.4 PATHOPHYSIOLOGY OF CIN

Radio-opaque CM are concentrated tri-iodinated benzene compounds that have an associated iodine moiety. By their nature, all CM compounds have cytotoxic effects related to the ionic strength, osmolality, or viscosity of the agent<sup>78</sup>. The first CM agents to be utilised were ionic ‘hyper-osmolar’ solutions however these have been withdrawn due to significant nephrotoxicity<sup>79</sup>. Safer non-ionic ‘low-osmolar’ (LOCM) and ‘iso-osmolar’ (IOCM) solutions were developed although these compounds are significantly more viscous than blood plasma<sup>80</sup> (Table 8). It is believed that the physicochemical properties of CM, in addition to additional vasoconstrictive and cytotoxic effects, are causative in CIN<sup>81</sup>.

The tissues of the kidney that exist under high metabolic and osmotic stress, supplied by a delicate microvascular circulation, are at high risk of ischaemic injury. This is particularly pertinent within the outer renal medulla where active sodium resorption in the ascending loop of Henle requires large amounts of oxygen despite a very low partial pressure of oxygen (10-20mmHg<sup>82</sup>), a result of poor tissue perfusion from the descending vasa recta (DVR), which is a vessel with a high vascular resistance commonly compromised by arterio-venous shunting<sup>83</sup>. In CKD, the greater metabolic burden placed upon a reduced number of functional nephrons, commonly supplied by a diseased micro and macro-vascular circulation, lowers the threshold for renal ischaemic injury further more<sup>84</sup>.

A comprehensive pathophysiological model of CIN remains the focus of ongoing research, however ischaemia in the outer medullary region of the kidney is believed to be a critical factor<sup>83</sup>. The cytotoxicity of CM has been shown to induce the vascular endothelium to release local vasoactive substances, including nitrous oxide (NO)<sup>85</sup>, adenosine, endothelin<sup>86</sup>, prostaglandins and reactive oxygen species (ROS) which cause profound vasoconstriction in critical vessels such as the DVR<sup>87</sup>. The onset of renal ischaemia in turn leads to a further release of vasoactive substances and a prolonged period of vasoconstriction when tissue damage is observed.

Compounding this, the greater viscosity of the CM and blood mixture within the DVR results in reduced medullary blood flow<sup>88</sup> and capillary obstruction may occur as a result of hyperosmolar red cell distortion and aggregation<sup>89</sup>. On restoration of normal vascular tone and blood flow, reperfusion of the ischaemic tissue is believed to exacerbate the tissue injury mediated via a number of complex pathways which will be the focus of discussion in following sections. Finally, CM is concentrated within the tubular filtrate to a degree where it may cause hyper-viscous tubular obstruction and a cytotoxic release of ROS resulting in acute tubular necrosis (ATN)<sup>90</sup>(Figure 1).

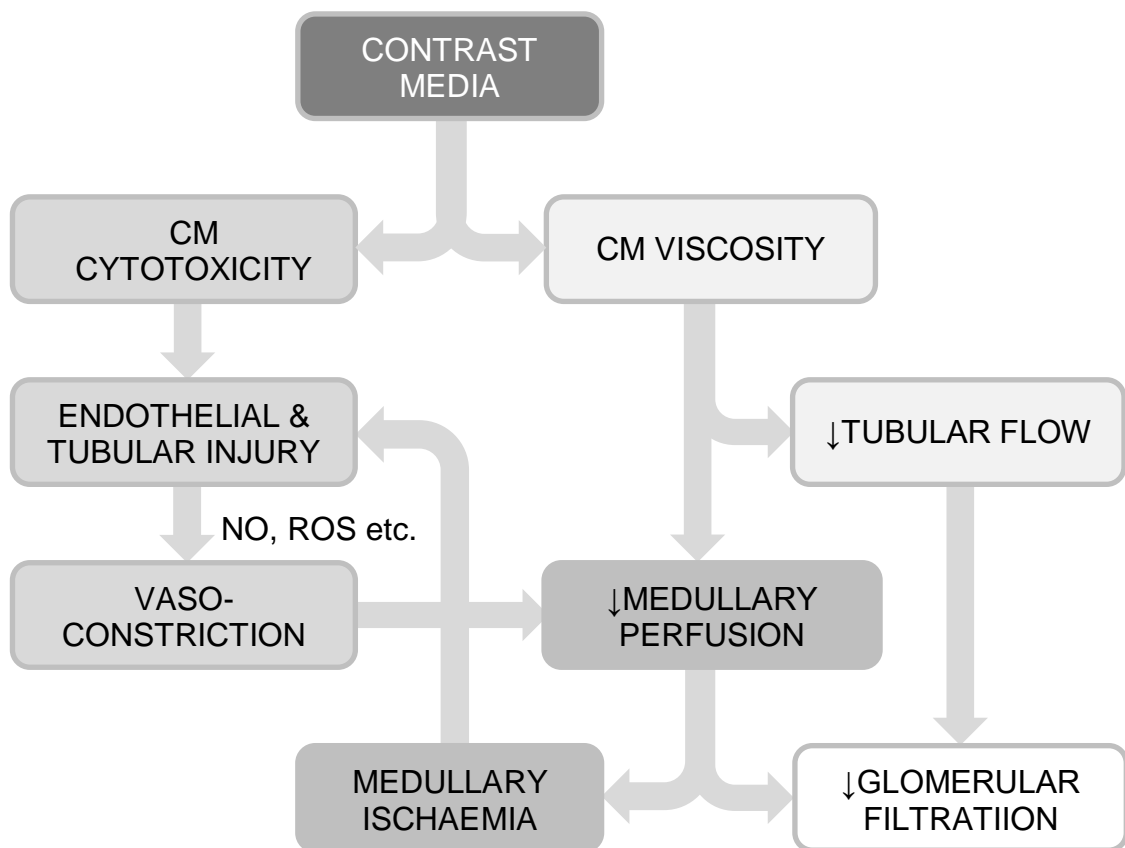
Elderly patients and those with diabetes or CKD, are at an increased risk of CIN due to inherent endothelial dysfunction causing an exaggerated vasoconstrictive response to CM<sup>91</sup>. Patients with congestive cardiac failure (CCF), reno-vascular disease, dehydration and hypotension are also at increased risk of CIN due the additional effect of renal vasoconstriction on outer medullary blood flow in the context of low renal preload. If the oxygen carrying capacity of blood is also reduced, as seen in anaemia or hypoxia, any reduction in renal blood flow will compound the ischaemic injury<sup>92</sup>.

**Table 8: Comparison of CM agents by osmolality and viscosity<sup>93</sup>**

	Blood Plasma	Iso-Osmolar Visipaque	Low-Osmolar Omnipaque	High-Osmolar Hypaque
<b>Osmolality mosmol/L</b>	290	290	844	2076
<b>Viscosity, 37° Centipoise</b>	3-4	11.8	10.4	8.4
<b>CIN risk</b>	-	Low	Low	High

CM = Contrast Media, CIN = Contrast Induced Nephropathy

**Figure 1: Proposed pathological mechanism of CIN**



CM = Contrast Media, NO = Nitric Oxide, ROS = Reactive Oxygen Species  
Adapted from Seeliger et al, Eur Heart J 2012<sup>94</sup>

### **1.3.5 RISK FACTORS FOR CIN**

An individually tailored CIN risk assessment including review of the indications for any CM based investigation should be reviewed prior to any procedure involving CM. Pre-procedural CIN risk factors are obtainable from the clinical history, physical examination and usual laboratory investigations and additional risk factors may be estimated and identified peri-procedurally.

#### **1.3.5.1 Chronic Kidney Disease**

The European Society of Urogenital Radiology (ESUR) Consensus Working Panel<sup>95</sup> in 1999 stated that “CIN risk becomes clinically significant when baseline SCr concentration is  $\geq 1.3$  mg/dL ( $\geq 115$  mmol/L) in men and  $\geq 1.0$  mg/dL ( $\geq 88.4$  mmol/L) in women”. This approximates to CKD stages III - V (when eGFR is  $< 60$  mL/min/1.73m<sup>2</sup>) and is recognised as the most important clinical risk factor for CIN<sup>96</sup>. Although this common biochemical investigation provides a cost effective screening tool, other risk factors are highly contributory to CIN, with many cases of CIN occurring in patients without pre-existing CKD<sup>45</sup>.

### 1.3.5.2 Diabetes Mellitus and acute hyperglycaemia

During the 1970's diabetes mellitus (DM) was recognised as an independent risk factor for the development of CIN<sup>97</sup>. This has since been convincingly demonstrated in a number of clinical trials<sup>52, 98</sup>, with significantly increased risk observed when co-existent with CKD<sup>99</sup>. The pathophysiological basis for increased susceptibility to renal ischaemia-reperfusion injury (IRI) in patients with DM can be explained by a number of factors: the presence macro and microvascular atherosclerotic disease affecting the renal vasculature, endothelial dysfunction and imbalance of vasoactive mediators, chronic tubulo-interstitial changes and regional hypoxaemia, increased oxygen demands due to additional tubular transport activity as well as increased generation of reactive oxygen species<sup>100</sup>.

In non-diabetic patients presenting with MI pre-procedural elevation of blood glucose prior to CM exposure has recently been shown to increase the risk of CIN in a large retrospective study performed by Alpert et al<sup>101</sup>. Interestingly there was no association found between CIN and pre-procedural glucose levels in diabetic patients, even after adjustment for confounding factors. Importantly the relationship between acute hyperglycaemia and CIN in non-diabetic patients persisted after accounting for disease severity indicators such as infarct size, which suggested that hyperglycaemia was not simply a marker of stress response to acute cardiovascular compromise and thus indirectly associated with CIN. The mechanism for this phenomenon remains unclear although may be related to acute hyperglycaemia mediated endothelial dysfunction<sup>102</sup>, increased oxidative stress<sup>103</sup> and decreased levels of nitric oxide<sup>104</sup>. However the results may also be attributable to unrecognised pre-existent diabetes in the non-diabetic cohort, less aggressive acute blood sugar control and lack of appropriate pre-hydration in these patients, all known factors that increase the risk of CIN.

### 1.3.5.3 Congestive cardiac failure (CCF)

CCF is a complex syndrome that results from failure of the heart to adequately perfuse the tissues, either due to functional or structural abnormalities of the myocardium, cardiac valves or pericardium that leads to disruption of the systolic and/or diastolic phase of the cardiac cycle. Patients with moderate to severe CCF symptoms (classified according to New York Heart Failure Association (NYHA) III or IV), a recent history of pulmonary oedema<sup>52</sup>, MI<sup>49</sup>, or those with a left ventricular ejection fraction (LVEF) of less than 45% are at increased risk of developing CIN<sup>98, 105</sup>. A number of complex pathological processes and clinical factors are responsible for the increased risk of CIN in this patient cohort.

Due to the overlap of aetiological factors in both renal and cardiac disease, as well as the complex interactions inherent in the cardio-renal syndrome, CKD and CCF often co-exist, significantly compounding the risks of CIN. Despite severe CKD commonly being an exclusion criterion in the landmark CCF trials, observed CCF/CKD co-prevalence rates were between 32% and 50% (Table 9). In addition, many of the cornerstone medications used in CCF, such as diuretics, ACE inhibitors and aldosterone antagonists may lead to hypovolaemia and hypotension resulting in renal hypo-perfusion. Clinicians may also be disinclined to prescribe prophylactic intravenous fluid to patients with CCF prior to CM exposure, due to the risk of precipitating an acute decompensation of CCF.



**Table 9: Prevalence of CKD in landmark CCF trials<sup>106</sup>**

<b>Study</b>	<b>Treatment</b>	<b>Exclusion SCr (<math>\mu\text{mol/l}</math>)</b>	<b>CKD prevalence eGFR&lt;60ml/min/1.73m<sup>2</sup></b>
SOLVD <sup>107</sup>	Enalapril	>177	32%
CIBIS-2 <sup>108</sup>	Bisoprolol	>300	33%
CHARM <sup>109</sup>	Candesartan	>265	36%
CARE-HF <sup>110</sup>	CRT	N/A	50%

Adapted from “Implications of kidney disease in the cardiac patient”, Rear et al, Interventional Cardiology Clinics, 2014.

#### 1.3.5.4 Advanced age

Advanced age, specified as age >75 years, is independently associated with an increased risk of CIN<sup>52</sup>, and is an important contributor to the increasingly frequent incidence of CIN, due to the cohort of cardiovascular patients generally being more elderly and the high number of CM based procedures now performed on these patients. In animal models and in humans, advanced age itself is associated with a physiological reduction in the total number of glomeruli, vascular morphological and functional abnormalities, glomerulo-sclerosis and interstitial fibrosis which are together responsible for the progressive age related fall in GFR and an increased risk of AKI<sup>111</sup>.

The cumulative exposure to disease processes affecting the kidney, including hypertension, diabetes and vascular disease as well as CCF, anaemia and nephrotoxic medications also significantly increases the incidence of CIN in the elderly. In addition, due to reductions in muscle bulk found in this population, measurement of SCr alone may not alert the clinician to the presence of underlying CKD unless formal eGFR calculation is performed<sup>112</sup>.

### **1.3.5.5 Female gender**

Although female patients are traditionally considered to be at higher risk of CIN than men, this is often attributed to the presence of confounding factors. However a large retrospective analysis suggested that women over the age of 65 years are indeed at greater risk of CIN than men of the same age with similar co-morbidity<sup>113</sup>. One explanation is that men have both larger and more numerous glomeruli than similar aged women, which is likely to be protective against renal IRI<sup>114</sup>. In addition females undergoing CA or PCI have been shown to have a higher incidence of CKD (74% vs 45%)<sup>115</sup>, anaemia and a higher risk of vascular and bleeding complications<sup>92</sup>, all known risk factors for CIN. Hormonal differences, including lower intramedullary prostaglandin production and increased platelet aggregation<sup>115</sup> may also be responsible for the observed increased CIN risk observed in women.

### **1.3.5.6 Anaemia**

Anaemia, defined as a haematocrit (HCT) of less than 0.39 in males or 0.36 in females is known to increase the risk of CIN; as demonstrated in a large registry study by Nikolsky et al<sup>92</sup>. Baseline anaemia was shown to be an independent predictor of CIN in patients with and without CKD (23% and 11% respectively). Patients with the lowest eGFR and HCT were found to be at highest risk (28.8%), however interestingly those patients with similarly low eGFR but normal HCT were relatively less vulnerable (15.4%). A peri-procedural fall in HCT of >5.9% was also shown to almost double the incidence of developing CIN (38.9%). This was most commonly due to acute haemorrhage either from traumatic vascular injury or from gastrointestinal blood loss attributable to anti-platelet and anti-thrombin therapy. Renal hypo-perfusion secondary to acute volume loss as well as the reduced oxygen carrying capacity of blood are likely to further compound medullary hypoxaemia and thus CIN.

### 1.3.5.7 Nephrotoxic medications

Co-administration of CM in the presence of nephrotoxic agents with direct tubular toxicity effects (Table 10) is thought to increase the risk of CIN, although this is variably documented in clinical studies<sup>116</sup>. Withdrawal of these agents, where clinically appropriate, for 24 hours prior to CM exposure is recommended. A particular area of interest pertains to concurrent treatment with angiotensin converting enzyme inhibitors (ACE-I) and Angiotensin receptor blockers (ARB's). Although not nephrotoxic per se, some small RCT's have reported that withholding ACE-I or ARB prior to CM exposure reduces the rate of CIN<sup>117</sup>, whereas other studies have not found any beneficial effect<sup>118</sup>. Current guidelines recommend that if these medications are part of established therapy, continuation is considered safer than the risk of withdrawal and that if initiation of ACE-I or ARB therapy is being considered this should be delayed until after CM exposure and CIN has been excluded<sup>96</sup>.

**Table 10: Nephrotoxic medications requiring withdrawal prior to CM exposure**

Drug Class	Examples
<b>Non-Steroidal Anti-Inflammatory drugs (NSAIDs)</b>	Naproxen, Ibuprofen, Diclofenac  Celecoxib <sup>119</sup>  Non-selective risk > Cox-2 selective risk
<b>Antibiotics</b>	Aminoglycosides: Gentamycin, Amikacin <sup>120</sup>
<b>Antifungal drugs</b>	Amphotericin B <sup>121</sup>
<b>Antiviral drugs</b>	Acyclovir, Tenofovir, Foscarnet <sup>122</sup>
<b>Immuno-modulatory drugs</b>	Cyclosporin A <sup>123</sup>
<b>Anti-neoplastic chemotherapy agents</b>	Cisplatin, Ifosfamide, Mitomycin <sup>124</sup>

### 1.3.5.8 Procedural factors

The total volume of CM administered is one of the most important risk modifiable factors in the development of CIN and has been shown to be the leading independent predictor for emergency dialysis<sup>125</sup>. In patients with CKD, administration of more than 125-140 ml of CM results in a 5-10 fold increase in CIN, irrespective of preventive measures<sup>74</sup>. Current ESC guidelines recommend limitation of contrast volume to 3 ml/kg. More specifically, Laskey et al<sup>126</sup> have identified that the maximum safe volume of contrast is dependent a ratio of the volume of contrast media to creatinine clearance (V/CrCl) of less than 3.7:1.

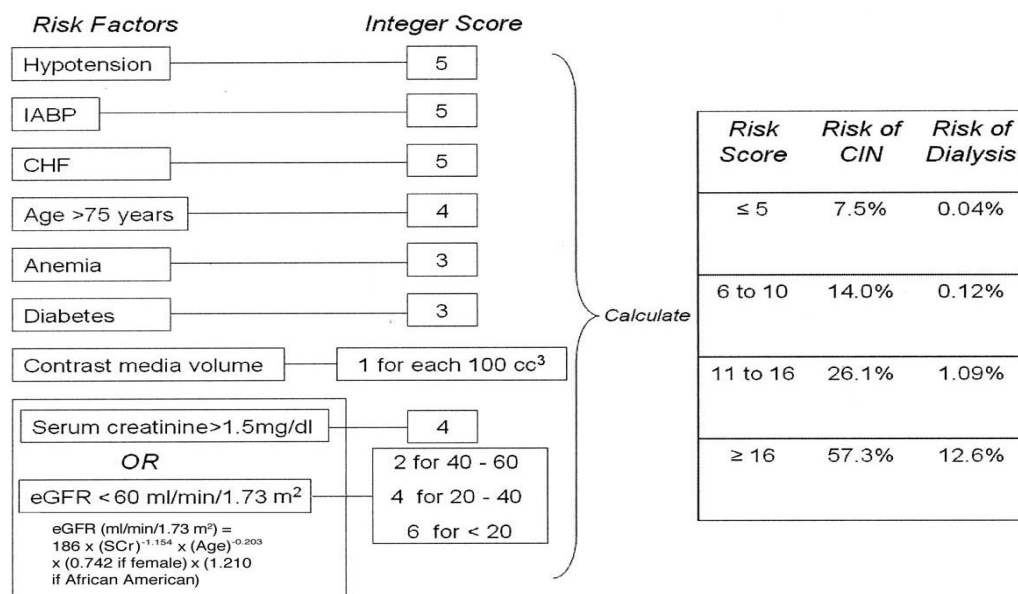
Other procedural factors such as total previous CM exposure within 72 hours<sup>51</sup> are directly related to the development of CIN. In addition the presence of peri-procedural haemodynamic instability, defined as a systolic blood pressure below 80mmHg for more than 60 minutes, use of inotropic agents or intra-arterial balloon pump (IABP)<sup>52</sup> therapy are all high risk factors. A recent meta-analysis failed to demonstrate any difference between rates of CIN following CM administered via the intra-arterial (IA) route as against the intravenous (IV) route, despite the long held assumption that an IA bolus might have a greater potential for toxicity<sup>127</sup>.

### 1.3.6 CIN RISK ASSESSMENT TOOLS

A commonly used CIN risk scoring system has been validated in a large cohort study by Mehran et al that incorporates weighted pre-procedural and peri-procedural risk factors<sup>52</sup> (Figure 2). Tziakas et al<sup>128</sup> have also developed a similar tool, albeit with a slightly different set of variables, including metformin use, previous PCI, peripheral arterial disease and  $\geq$  300 ml of CM. Both scoring systems are limited by estimation of CIN risk only after CM has been administered, when there is a clear clinical need to predict CIN pre-procedurally when prophylactic measures are most effective. As such, a pre-procedural CIN risk score has been validated in a prospective cohort by Maioli et al<sup>51</sup> (Table 11).

Several novel CIN risk factors have recently been identified, such as pre-procedure serum glucose<sup>129, 130</sup> and LDL-C<sup>131</sup> however these have not yet been integrated into a validated risk-scoring tool. Commonly used cardiovascular risk scoring systems, such as the Global Registry of Acute Coronary Events (GRACE) score in AMI patients, have been shown to predict risk of CIN in patients with normal renal function and a GRACE inpatient risk score of greater than 140 ( $>3\%$  mortality)<sup>132</sup>. Bio-Impedance Vector Analysis (BIVA), an investigative measure of total body hydration, has also been shown to correlate with the risk of developing CIN in a proof of concept clinical trial<sup>133</sup> and shows promise of being incorporated into a CIN risk scoring system and guided hydration strategy.

**Figure 2: The Mehran CIN risk score<sup>52</sup>**



Hypotension = SBP < 80 mmHg > 1hr / inotropic support, IABP = IABP within 24hrs of procedure, CHF = NYHA III-IV or history of pulmonary oedema, Anaemia = Male: HCT < 0.39, Female: HCT < 0.36

**Table 11: A Pre-procedural Risk score for CIN, adapted from Maioli et al<sup>51</sup>**

Pre-Procedural Risk Factor		Score		
Prior CM exposure within 72 hours		3		
Left Ventricular Ejection Fraction < 45%		2		
Pre-procedure SCr > Baseline SCr		2		
Baseline SCr > 1.5mg/dl		2		
Diabetes Mellitus		2		
Creatinine Clearance (eGFR) < 44ml/min		2		
Age > 73 years		1		
Score	0 - 3	4 - 6	7 - 8	> 9
CIN risk	Low 1.1%	Moderate 7.5%	High 22.3%	Very High 52.1%

CM = Contrast Media, SCr = Serum Creatinine, eGFR = Estimated Glomerular Filtration Rate



### **1.3.7 PREVENTATIVE STRATEGIES AGAINST CIN**

The following sections will summarise recent evidence based guidelines that provide a framework for the prevention of CIN, as published in the European guidelines (ESC<sup>134</sup>, ESUR<sup>96</sup>), national (NICE) and local (UCLH) guidelines. Optimisation of the patients circulating volume prior to and during CM exposure remains the most effective single intervention, although the strategy for achieving this remains controversial<sup>135</sup>. In addition a number of other important considerations and interventions have been shown to be effective.

#### **1.3.7.1 CIN Risk Assessment**

A CIN risk assessment should be performed on all patients referred for CM based investigations, including baseline measurement of SCr and calculation of eGFR using an appropriate formula (e.g. Modification of Diet in Renal Disease (MDRD)). Patients that are identified as being at increased risk of CIN should be considered for a non-CM based procedure if clinically acceptable. If a 'follow on' CM procedure is necessary after initial CM exposure, this should ideally be delayed until adequate clearance of CM or normalisation of renal function has occurred, in non-urgent cases after two weeks or in urgent cases as long as is clinically appropriate.

Due to mutual risk factors and interaction between CKD and cardiac disease, patients identified as being at risk of CIN are also at higher risk of poor cardiovascular outcomes and thus clinician concern about the risk of CIN should not prevent or unnecessarily delay prognostic CM based procedures.

### 1.3.7.2 Pre-procedural medication review

All patients should discontinue non-essential nephrotoxic medications (Table 10) as well as loop diuretics for 24 hours before and 48 hours after CM exposure, restarting these only once SCr measurement excludes CIN. Patients taking Metformin who receive intra-arterial CM with an  $eGFR < 60 \text{ ml/min/1.73m}^2$ , or who receive intra-venous CM with an  $eGFR < 45 \text{ ml/min/1.73m}^2$ , should ideally discontinue Metformin for 48 hours pre-procedure and restart once CIN has been excluded. Although Metformin is not nephrotoxic in itself, some studies have shown an increased risk of lactic acidosis following CIN although significant inconsistencies between studies mean that only a low level of evidence exists for this recommendation<sup>136</sup>. It is important to note that elevated pre-procedural serum glucose levels are themselves implicated in the development of CIN and so must be taken into account when considering Metformin withdrawal.

### 1.3.7.3 Choice of CM and radiographic considerations

Use of HOOCM agents are now contra-indicated in patients at risk of CIN, however it is less clear whether IOCM formulations are preferable over LOCM. A number of meta-analyses have been performed which present conflicting results<sup>93, 137</sup>. Current guidelines recommend the use of either IOCM or LOCM, as long as the amount of CM required for diagnostic accuracy is kept to a minimum and is under the threshold of 3ml/kg or a V/CrCl  $< 3.7:1$ . In experienced centres, the use of biplane imaging during CA may reduce CM volumes as simultaneous orthogonal views can be acquired<sup>138</sup>. Novel automated contrast injection (ACI) devices have also been shown to reduce the volume of CM used and may reduce the incidence of CIN<sup>139</sup>.

#### 1.3.7.4 Pre-hydration strategies

Ensuring that patients are adequately hydrated prior to CM exposure is the single most effective prophylactic measure to prevent CIN. Optimising intravascular volume maintains renal blood flow and dilutes CM in blood and tubular filtrate. In low risk ambulant patients oral hydration is acceptable if adequate fluid intake is possible. In higher risk patients or those unable to tolerate oral hydration, intravenous (IV) crystalloid is preferred as it guarantees appropriate fluids are delivered and is superior to oral hydration in clinical trials<sup>140</sup>.

There continues to be debate regarding which formulation of crystalloid is optimal, with some centres favouring intravenous 'normal saline' (0.9%) and others favouring intravenous sodium bicarbonate (1.26%) which is believed to be superior due both to its additional ROS scavenging ability<sup>141</sup> and lack of chloride ions that may exacerbate renal vasoconstriction<sup>142</sup>. Although some meta-analyses have suggested a slight reduction in CIN rates with sodium bicarbonate, no clear mortality benefit has been demonstrated<sup>143, 144</sup>. Owing to a considerable cost difference between the two therapies, many centres continue to favour the use of normal saline. However, one practical advantage of sodium bicarbonate is that most studies demonstrated equivalence to normal saline with shorter administration times and a lower fluid volumes, which may be useful for elective patients and those who are unable to tolerate large amounts of i.v. fluid, such as elderly patients and those with CCF<sup>145</sup>. (Table 12)

**Table 12: Intravenous pre-hydration regimes, updated ESUR guidelines 2011<sup>96</sup>**

<b>IV Fluid</b>	<b>Pre Hydration</b>	<b>Post Hydration</b>
<b>Isotonic Saline (0.9%)</b>	6-12 Hours, 1-1.5ml/Kg/Hr	6-12 Hours 1-1.5ml/Kg/Hr
<b>Sodium Bicarbonate (1.26%)</b>	1 Hour 3ml/Kg/Hr	6 Hours 1ml/Kg/Hr

### **1.3.7.5 Pharmacological prophylaxis against CIN**

Few pharmacological agents have proven efficacy against CIN, however N-Acetyl-Cysteine (NAC), an inexpensive and well tolerated antioxidant and vasodilator, given at dose of 600mg BD for 24 hours pre and post procedure has been shown to reduce the incidence of CIN in several large RCT's<sup>146, 147</sup>. Despite these findings, recent meta-analyses<sup>148, 149</sup> have been equivocal, most likely as a result of wide heterogeneity, variable reporting and publication bias in the included studies<sup>150</sup>. As such it is not recommended that NAC is used alone, although it may be a useful additional agent and is rarely harmful when added to conventional therapy in high-risk patients<sup>151</sup>.

### **1.3.7.6 Post procedure renal monitoring and management of AKI**

All patients at risk of CIN should have repeat SCr levels measured from 48 to 72 hours following CM exposure. In addition those at very high risk should have urinary output measured, often requiring insertion of an indwelling urinary catheter connected to an urometer. If CIN occurs (see Table 6) then recommended AKI management guidelines, such as included in the recent European Best Practice position statement on AKI<sup>151</sup>, should be implemented. This consists of serial SCr measurements, withholding nephrotoxic medications and loop diuretics, optimisation of electrolyte and hydration status, nutritional input, and if severe early hospitalisation with input from specialist nephrology teams.

### **1.3.8 NOVEL PHARMACOLOGICAL PROPHYLAXIS AGAINST CIN**

A number of pharmaceutical agents with antioxidant and vasodilatory properties have been investigated and although some have shown promise, further evaluation is required (Table 13). The three agents with the most supportive evidence are theophylline/aminophylline, high dose statins and ascorbic acid.

#### **1.3.8.1 Theophylline & Aminophylline**

The use of competitive adenosine antagonists, such as theophylline and aminophylline, as prophylaxis against CIN stems from the rationale that inhibiting adenosine mediated renal vasoconstriction (section 1.4.2.4) should offer protection against renal IRI. Theophylline and aminophylline are also non-specific competitive phosphodiesterase inhibitors which may also contribute to reno-protection through anti-inflammatory pathways. Initial evidence for efficacy was limited, as shown in an early meta-analysis by Ix et al, who demonstrated a small difference in post procedure mean SCr between groups (11.5  $\mu\text{mol/l}$ ). A larger and more recent meta-analysis by Dai et al<sup>152</sup>, consisting of 1412 patients in 14 RCT's, has found a more pronounced effect with an overall CIN relative risk (RR) of 0.48 (95% CI 0.26-0.89, P=0.02) and a significant reduction in post procedure SCr in the treatment group (27.4  $\mu\text{mol/l}$ ). Nevertheless the included studies showed significant heterogeneity and so no firm conclusions can be made until further large RCT's are performed.

### 1.3.8.2 High dose Statins

Hydroxymethylglutaryl CoA reductase inhibitor (statin) therapy is an important component in the treatment of ACS and CVD which significantly improves both short and long term cardiovascular outcomes<sup>153</sup>, particularly so when initiated prior to PCI<sup>154</sup> and at high doses<sup>153</sup>. This effect is mediated via lipid profile optimisation as well as by pleiotropic effects including improvements in endothelial function<sup>155</sup>, anti-inflammatory<sup>156</sup> and anti-oxidative properties<sup>157</sup> and inhibition of thrombin formation and platelet aggregation<sup>158</sup>.

The recent PRATO-ACS study<sup>159</sup> demonstrated that high dose rosuvastatin pre-treatment (40mg then 20mg od) in statin naïve ACS patients was protective against CIN (6.7% vs. 15.1%; OR 0.38; 95% CI 0.20 - 0.71;  $p = 0.003$ ) in addition to improving cardiovascular outcomes. A follow up meta-analysis by Marenzi et al<sup>160</sup>, including 5212 patients in 9 RCT's, conformed reduction in the rate of CIN when treating statin naïve ACS patients (5.5% vs 15%, RR 0.37; 95% CI 0.25 to 0.55;  $P < 0.0001$ ) however this benefit did not extend to non-ACS patients, although there was a non-significant trend towards benefit. Importantly another meta-analysis by Thompson et al<sup>161</sup> did not show benefit in treating statin naïve patients with stage III CKD, although this may be explained by the limited number of trials that specifically include this group of patients. As such further specifically designed RCT's are required to elucidate whether statin pre-treatment can reduce the incidence of CIN in high-risk patients.

### 1.3.8.3 Ascorbic Acid

The rationale of antioxidant therapy in CIN prophylaxis is derived from the evidence supporting NAC and the role that ROS is believed to play in CIN (section 1.4.2.9). Ascorbic acid (vitamin C) is well tolerated, has powerful antioxidant properties and was therefore proposed as a potentially beneficial agent. However despite promising data supporting its protective role against ischaemic AKI in animal models<sup>162</sup>, clinical trials have been less conclusive. An initial pilot RCT performed by Spargias et al<sup>163</sup> in 2004 demonstrated that pre-treatment with high dose ascorbic acid reduced the incidence of CIN (9% vs 20% OR 0.38, 95% CI 0.17 to 0.85; P=0.02). However a number of other further RCT's failed to demonstrate benefit<sup>164, 165,166</sup>. Nevertheless a recent meta-analysis of 9 small RCT's, performed by Sadat et al<sup>167</sup>, found that ascorbic acid pre-treatment may reduce CIN rates by up to 33%, with no significant evidence of heterogeneity or publication bias in the studies examined. As such ascorbic acid remains an interesting therapy that warrants further investigation in large RCT's.



**Table 13: Novel pharmacological prophylaxis against CIN**

<b>Drug name</b>	<b>Study</b>	<b>Outcome: Treatment vs Control</b>
<b>High dose Statins</b>	PRATO-ACS <sup>159</sup> N=504 RCT	CIN 6.7% vs. 15.1% OR 0.38; 95% CI 0.20 - 0.71; p = 0.003
	Marenzi et al <sup>160</sup> N= 1134	CIN 5.5% vs 15% RR 0.37; 95% CI 0.25 to 0.55; P<0.0001
<b>N-Acetyl Cysteine</b>	Gonzales et al <sup>148</sup> Meta-analysis N=2746 RCT	High quality RCTs - no CIN benefit RR=0.87; 95% CI 0.68-1.12, p = 0.28 Low quality RCTs – high CIN benefit RR=0.15; 95% CI 0.07-0.33, p < 0.0001
	<b>Limited Evidence</b>	
<b>Ascorbic Acid</b>	Sadat et al <sup>167</sup> , Meta-analysis N=1561, 9 RCT	CIN 33% reduction RR 0.672; 95% CI 0.466 to 0.969; p = 0.034
<b>Theophylline</b>	Ix et Al <sup>168</sup> , Meta-analysis N= 480 RCT	Difference in mean SCr 11.5 µmol/l 95% CI 5.3-19.4 µmol/l, p = 0.004
	Dai et al N= 1412 RCT	Reduction in CIN (Heterogeneity) RR 0.48, 95% CI 0.26-0.89, P=0.02
<b>Iloprost</b>	Spargias et al <sup>169</sup> N=208, RCT	CIN 8% vs 20% OR 0.29 95% CI 0.12 to 0.69; P=0.005
<b>Atrial natriuretic peptide</b>	Morikawa et al <sup>170</sup> N=254, RCT	CIN 3.2% vs. 11.7% OR 0.24; p = 0.016
<b>Trimetazidine</b>	Shehata et Al <sup>171</sup> N=100, RCT	CIN 12% vs 28% p <0.05
<b>Prostaglandin E1</b>	Li et al <sup>172</sup> N=163, RCT	CIN 3.7 vs. 11.1 % P < 0.05
<b>Negative or conflicting evidence:</b> Fenoldopam <sup>173</sup> , dopamine <sup>174</sup> , calcium channel blockers <sup>175</sup> , L-arginine <sup>176</sup> , furosemide <sup>177</sup> , mannitol <sup>178</sup> , endothelin receptor antagonists <sup>179</sup> .		

CIN = Contrast Induced Nephropathy, RCT = Randomised Controlled Trial, RR = Relative Risk, CI = Confidence Interval, OR = Odds Ratio, SCr= Serum Creatinine, PCI = Percutaneous Coronary Intervention, CKD = Chronic Kidney Disease, DM = Diabetes Mellitus

### 1.3.9 NOVEL INTERVENTIONAL PROPHYLAXIS AGAINST CIN

Novel interventional therapies may offer further protection when combined with standard therapy and can broadly be characterised into three areas: hydration optimisation, CM extraction and renal protection. Although numerous small studies investigating novel CIN prophylactic strategies have shown promise, no intervention has as yet been proven to be effective and successfully translated into clinical practice.

#### 1.3.9.1 Novel hydration strategies

The optimal volume and rate of i.v. fluid delivery is often difficult to quantify and whilst under-hydration increases CIN risk, over hydration may precipitate acute pulmonary oedema in patients with severe CKD and CCF necessitating i.v. diuretic use. Two strategies for improving peri-procedural hydration have been investigated.

Left ventricular end diastolic pressure (LVEDP) guided volume expansion, using a cardiac catheter placed in the left ventricle to attain intra-procedural LVEDP measurements that determine the amount of i.v. fluid needed, has been shown to be a safe and effective treatment in the POSEIDON RCT<sup>180</sup>, with a reduced incidence of CIN from 16.3% with standard hydration to 6.7% in the LVEDP-guided group.

Creation of a 'forced diuresis' by administering a matched fluid and furosemide infusion has demonstrated efficacy in the MYTHOS study, reducing CIN from 18.6% to 4.6%<sup>181</sup>. Building upon these results, the RENALGUARD system maintains a high urine output (>300ml/hr) using balanced i.v. isotonic saline (0.9%) and i.v. Furosemide infusion (0.25mg/kg), plus oral NAC. In the REMEDIAL II study<sup>182</sup> this reduced CIN to 11%, (16/146) from 20.5% (30/146) in the i.v. Sodium Bicarbonate plus oral NAC control group (OR 0.47; 95% CI 0.24 - 0.92).

### **1.3.9.2 Novel CM removal strategies**

Removal of CM from the blood pool should in theory show benefit in preventing CIN, however prophylactic haemodialysis (HD) after CM procedures has not been shown to reduce CIN<sup>183</sup>, whereas both pre and post procedural hemofiltration (HF)<sup>184</sup> and simultaneous HF<sup>185</sup> have shown limited efficacy, possibly solely due to optimisation of peri-procedural intravascular volume. HF is a costly intervention with associated risks which should therefore be reserved for those patients at the highest risk of CIN, for example in those with end stage renal disease (ESRD)<sup>186</sup>. In order to minimise the amount of CM that perfuses the renal vasculature, catheter devices have been developed to filter and extract CM from coronary sinus venous blood, which contains high concentrations of CM during CA, before it mixes back into the general circulating blood pool<sup>187</sup>, however this emerging technology has yet to be translated into clinical trials.

### **1.3.9.3 Novel reno-protective strategies**

Remote ischaemic conditioning (RIC), a prototypical treatment against ischaemia reperfusion injury (IRI) which is pathognomic to CIN, has been shown to be effective in animal and clinical studies<sup>188</sup>. Several proof of concept RCT's have demonstrated a 60-70% reduction in CIN using cycles of brief, non-injurious remote tissue ischaemia induced by blood pressure cuff inflation on the upper arm prior to CM exposure (preconditioning/RIPC)<sup>189</sup>. Additionally, brief cycles of myocardial ischaemia induced by catheter balloon inflation in the target coronary artery following PCI may also reduce CIN after CM exposure (remote ischaemic post-conditioning/RIPostC)<sup>190, 191</sup>. RIPC presents a novel reno-protective prophylactic strategy that will be examined in depth in the following sections and is the focus of the clinical trial presented within this thesis.

## 1.4 ISCHAEMIA-REPERFUSION INJURY

Ischaemia-reperfusion injury (IRI) occurs following injurious reduction in arterial perfusion and oxygenation of an organ (ischaemia) followed by restoration of blood flow and re-oxygenation of the ischaemic tissues (reperfusion). Organs that are most at risk are those with high oxygen demand and metabolic activity, most notably the heart, kidney, liver, intestine and brain. Clinical scenarios in which IRI occurs involve acute compromise of an organ's blood supply, as a result of processes such as atheromatous plaque rupture and thrombosis, vascular dissection, embolism or vasoconstriction, followed by reperfusion of the affected tissues either by spontaneous reflow or following medical intervention, typified in conditions such as MI, CVA and CIN.

Renal IRI typically occurs following injurious systemic hypo-perfusion in conditions such as cardiogenic shock, sepsis, major haemorrhage and anaphylaxis and is a component of the systemic inflammatory response syndrome (SIRS)<sup>192</sup> and multi organ dysfunction syndrome (MODS)<sup>193</sup>, responsible for between 30% and 40% of mortality on intensive care units. It is also a common complication during invasive therapies such as PCI (CIN), CABG surgery (cardiopulmonary bypass), vascular surgery (aortic cross-clamping) and renal transplantation. The development of ESRD and treatment with regular haemodialysis exposes patients to recurrent hypovolaemic stress affecting cardiac<sup>194</sup>, cerebral<sup>195</sup> and enteric<sup>196</sup> perfusion contributing to the increased morbidity and mortality seen in this patient group<sup>197</sup>.

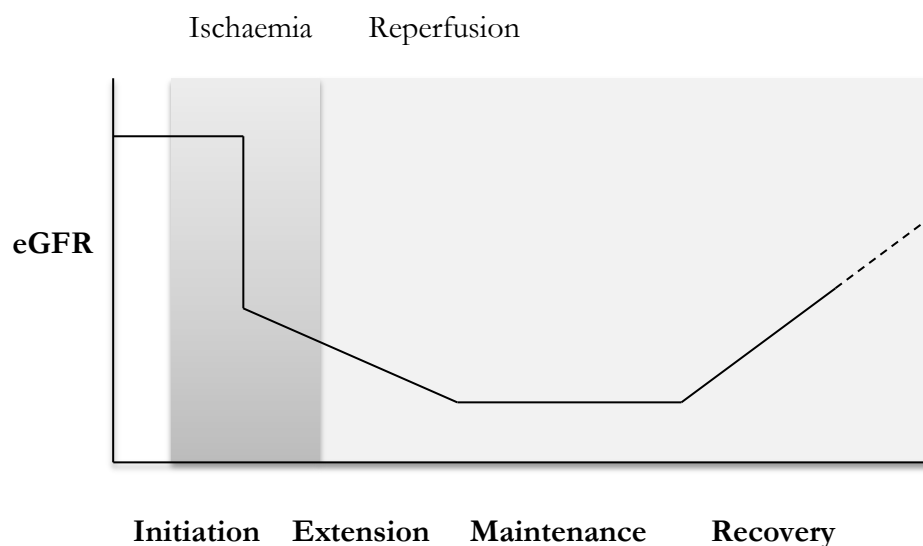
The intra-cellular mechanisms involved in IRI are complex and not yet fully understood, however a number of proposed pathways will be discussed in depth in the following sections. In general the cellular injury that occurs in IRI may be as a consequence of reperfusion triggered cell death and/or an event that occurs during ischaemia that manifests during the reperfusion process<sup>198</sup>.

Myocardial IRI has been the subject of extensive investigation, however the mechanisms of IRI in the kidney will be the focus of the following sections. In particular, due to the pivotal role that IRI is believed to play in CIN, specific prophylactic therapies that are believed to ameliorate CIN through this pathway will be explored.

#### 1.4.1 ISCHAEMIA-REPERFUSION INJURY OF THE KIDNEY

IRI of the kidney, either due to pre-renal hypo-perfusion (hypovolaemia, cardiogenic shock and major surgery) as well as due to CIN, leads to acute tubular necrosis (ATN) and reduction in GFR, the hallmark of AKI. ATN can be clinically and temporally divided into four stages; initiation, extension, maintenance and recovery, which directly relate to cellular and histological pathological events (Figure 3).

**Figure 3: Four pathophysiological phases of ATN**



#### **1.4.1.1 Initiation phase**

Following injurious renal hypo-perfusion, the initiation phase of ATN is heralded by decreased oxidative phosphorylation of adenosine diphosphate (ADP) into adenosine triphosphate (ATP) within the mitochondria of the renal vascular epithelium, endothelium and smooth muscle cells. In the same way reductions in levels of Guanosine triphosphate (GTP) are observed. As a consequence, failure in a wide range of intracellular structural and functional processes occurs. ATP degradation leads to disruption of the intracellular framework of filamentous actin (F-Actin)<sup>199</sup> causing macroscopic loss of the renal brush border and disruption of cellular membrane proteins involved in cell signalling, epithelial polarity and tight junction formation. Up-regulation of vasoactive mediators, cytokines and chemokines occurs, leading to vasoconstriction and activation of the inflammatory cascade<sup>200</sup>.

#### **1.4.1.2 Extension phase**

Progression to the extension phase is potentiated by ongoing vasoconstriction of the renal vasculature. Lethal and sub-lethal tubular injury occurs which is particularly pronounced in the cortico-medullary junction (CMJ) and outer medullary region where tissues exist on the brink of hypoxia (10-20mmHg) under normal conditions. Vascular and tubular damage occurs due to persistent activation of the inflammatory cascade, leukocyte infiltration<sup>201</sup>, intracellular dysfunction and oxidative stress following reperfusion, leading to a precipitous fall in GFR.

### **1.4.1.3 Maintenance phase**

The maintenance phase occurs following established reperfusion that leads to initiation of cellular repair mechanisms, proliferation, migration and apoptosis of cells in an attempt to restore tubular integrity and intracellular homeostasis. GFR generally plateaus at this point, lasting from a few days up to two weeks. During this period the tubulo-glomerular feedback mechanism causes reduced renal blood flow due to vasoconstriction of the afferent renal arterioles in response to detection of an increased salt load in the distal tubules by macula densa cells. Uraemic complications are often seen during this phase and diuretic therapy may be required to manage salt and water balance.

### **1.4.1.4 Recovery phase**

The recovery phase involves re-establishment of normal cellular function, epithelial polarity and differentiation of cells, with recovery of GFR dependent on the degree of tissue injury sustained. Diuresis commonly occurs during this period, causing water and electrolyte loss and volume depletion, the mechanism of which is not completely understood, although delayed recovery of tubular function with increased GFR may account for this phenomenon.

## **1.4.2 PATHOPHYSIOLOGY OF RENAL IRI**

Multiple vasoactive mediators are involved in the initiation and extension phases of ATN that exert complex effects dependent on the location and subtypes of receptor within the kidney. The imbalance of vasoactive mediator production consequent to ischaemia of the vascular epithelium leads to prolonged medullary vasoconstriction and activation of the inflammatory cascade that precedes renal IRI.

### **1.4.2.1 Norepinephrine, Renin, Angiotensin II**

Systemic and renal hypo-perfusion is associated with a strong sympathetic neuronal and endocrine response consisting of release of norepinephrine and renin and activation of angiotensin II (ANGII). Increased sympathetic tone and release of norepinephrine, renin and ANGIID causes profound renal vasoconstriction; that compounds pre-renal hypo-perfusion and worsens renal ischaemia. Renal sympathetic denervation<sup>202</sup> has been shown to be protective against AKI in animal studies, however renin and angiotensin blockade does not seem to confer the same protective effect, with some clinical trials reporting a deleterious effect on CIN<sup>117</sup> and increased AKI following cardiac surgery<sup>203, 204</sup>.



### 1.4.2.2 Endothelin

Endothelin is a potent vasoconstrictive molecule that has been shown to be elevated following ischaemic AKI<sup>205</sup>. It interacts with both ET-A and ET-B receptors, activation of which results in reduced renal blood flow and GFR. Although a promising target for reno-protection, clinical trials suggest non-selective endothelin blockade appears to significantly exacerbate renal injury due to CIN<sup>179</sup>. The explanation for this is unclear, however it may be related to complex interactions with other vasoactive substances, differing specific function of ET-A and ET-B receptors or a preferential renal cortical response to endothelin causing a vascular 'steal' phenomenon affecting the vulnerable outer medullary region<sup>91</sup>.

### 1.4.2.3 Platelet-activating factor

Platelet activating factor (PAF) is a lipid mediator secreted by many organs including the kidney in response to ischaemia that causes concentration dependant renal vasoconstriction and reduction in GFR. PAF levels are also increased by release of endothelin and renin, which support its role in renal vasoconstriction<sup>206</sup>. PAF antagonists have been also shown to be protective against AKI in a number of animal studies<sup>207, 208</sup>. Despite this, PAF itself has been shown to cardio-protective against IRI, mediated through activation of mitochondrial  $K_{ATP}$  channels that are known to protect against cell death<sup>209</sup>.

#### 1.4.2.4 Adenosine

Impaired Na<sup>+</sup> resorption in the proximal tubule in response to ischaemia leads to activation of the tubulo-glomerular feedback (TGF) mechanism and the subsequent release of adenosine, which in turn causes potent afferent renal vasoconstriction mediated by A<sub>1</sub> receptors<sup>210</sup>. This effect is more pronounced in the renal cortical regions and may act to shunt blood toward the vulnerable medulla and reduce the metabolic load on the kidney, however prolonged cortical vasoconstriction, as seen in CIN, leads to tubular injury. Although adenosine mediated vasoconstriction might be expected to worsen ATN, animal studies suggest it plays a more complex role, including exerting a protective anti-inflammatory effect mediated through the A<sub>2a</sub> receptor<sup>211</sup>. Adenosine A<sub>1</sub>, A<sub>2b</sub> and A<sub>3</sub> receptors are involved in the trigger mechanism for activation of protein kinase C (PKC)<sup>212</sup> and the reperfusion injury salvage kinase (RISK) pathway, consisting of AKT and ERK1/2, which protect against cell death by preventing opening of the mitochondrial permeability pore (MPTP) (section 1.4.2.11).

#### 1.4.2.5 Prostaglandins

As a protective response to ischaemia, renal medullary cells secrete prostaglandins, including prostacyclin PGE<sub>2</sub>, which exert a strong vasodilatory effect on the renal vasculature as well as inhibiting production of intracellular adhesion molecules (ICAM), critical for leukocyte infiltration. The concomitant use of cyclooxygenase (COX) inhibitors, such as non-steroidal anti-inflammatory drugs (NSAIDs), strongly contributes to the risk of CIN<sup>213</sup>, likely mediated through the reduction in COX-2 dependant prostaglandin production in the kidney. In a recent small RCT<sup>169</sup>, the synthetic prostaglandin iloprost demonstrated protection against CIN in susceptible individuals.

#### 1.4.2.6 Nitric Oxide

Nitric oxide (NO), a potent vasodilator involved in autoregulation of renal blood flow, is produced by several isoforms of nitric oxide synthase (NOS) from L-arginine substrate. Endothelial NOS (eNOS) and neuronal NOS (nNOS) are found in particularly high concentrations in the renal vascular epithelium in the cortico-medullary region<sup>214</sup>, whereas inducible NOS (iNOS) is found predominantly in the glomerulus and renal tubular cells. Following AKI, eNOS activity is significantly reduced leading to unopposed vasoconstriction from other vasoactive substances such as endothelin and Angiotensin II<sup>215</sup>. However following renal ischaemia, iNOS activity increases and interestingly has shown to be detrimental to IRI in the kidney<sup>216</sup>. This may be explained by the formation of toxic metabolites, such as peroxynitrite, from reaction between excessive NO and reactive oxygen species (ROS) production within the tubular cells. Non-specific inhibition of NOS in renal IRI has been found to be detrimental<sup>217</sup>, likely due to the vasoconstrictive response to reduced eNOS activity, whereas specific inhibition of iNOS has been found to be protective in animal models<sup>218</sup>.

#### 1.4.2.7 Microvascular thrombosis

Endothelial dysfunction following renal IRI may lead to the formation of thrombotic micro-emboli within the renal microvasculature, which further exacerbates ischaemic damage. Intravascular thrombin and fibrin deposition have been described in animal models of renal IRI<sup>219</sup> and following renal transplantation in humans. In addition to reduced renal blood flow, the loss of cell surface inhibitory modulators such as thrombomodulin and activated protein C, with increased expression of pro-thrombotic glycoproteins such as tissue factor may explain this phenomenon<sup>220</sup>. Administration of soluble thrombomodulin has also been shown to ameliorate ischaemic AKI in animal models<sup>221</sup>

#### 1.4.2.8 Inflammatory cascade activation and leukocyte infiltration

Leukocyte adhesion to and infiltration across the renal vascular epithelium is characteristic of renal IRI and is seen as early as 1 hour following an ischaemic insult<sup>201</sup>. Accumulation of leukocytes within the vascular lumen may cause obstruction in addition to contributing to vasoconstriction via the secretion of vasoactive mediators. Once infiltrated across the vascular epithelium, leukocytes exert direct cytotoxicity on the renal parenchyma, with the ensuing interstitial oedema further reducing renal perfusion by restricting peritubular capillary flow and increasing tubular pressure. Endothelial expression of leukocyte adhesion molecules, such as ICAM and P-selectin for neutrophil/natural killer T-Cell infiltration and fractalkine for macrophage chemo-attraction, are an integral part of the inflammatory response to renal ischaemia.

Inactivation of these molecules using specific antibodies has shown some promise in ameliorating renal IRI in animal models<sup>222, 223</sup>. Once infiltrated these activated leukocytes, in addition to injured endothelial cells, are responsible for the production of large quantities of pro-inflammatory signalling molecules such as interferon gamma (IFN $\gamma$ ), tissue necrosis factor alpha (TNF $\alpha$ ), and interleukins (IL1, IL6, IL8, IL12, IL18) that further promote the cascade of inflammation<sup>224</sup> and apoptosis<sup>225</sup> both locally and systemically.

### 1.4.2.9 Reactive oxygen species generation

Excessive reactive oxygen species (ROS) generation, consisting primarily of superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical (HO) and peroxynitrite, occurs as a result of oxidative stress and leukocyte activation and has been identified as playing an important role in the pathogenesis of renal IRI<sup>226</sup>. ROS are highly reactive molecules that result from disturbances in intermediary metabolism, ischaemic blockade of the mitochondrial electron transport chain<sup>227</sup>, leukocyte NADPH oxidase activity<sup>226</sup> and catalysis of hydrogen peroxide into the hydroxyl radical by free iron.

During ischaemia NADPH dependent xanthine dehydrogenase, which is usually responsible for nucleic acid degradation, is converted into xanthine oxidase. Following re-oxygenation, xanthine oxidase acts upon excess hypoxanthine and xanthine accumulation resulting from ATP depletion and generates superoxide anion and hydrogen peroxide ROS. Excessive NO released as a result of ischaemia reacts with superoxide anion to form peroxynitrite, a particularly aggressive species of molecule. ROS cause widespread cellular damage by reacting directly with DNA, cell membrane lipids (lipid peroxidation), intracellular and cell membrane proteins and glycosaminoglycans as well as activating redox signalling.

Cellular antioxidant defence mechanisms, consisting of superoxide dismutase, catalase and glutathione-s-transferase, which maintain ROS at non-harmful levels during normoxaemia, are variably impaired during IRI, contributing to ROS induced cellular injury<sup>228</sup>. Supplementation with N-acetyl-cysteine, a glutathione precursor, has shown promise in animal models of AKI<sup>229</sup> however results from clinical trials investigating its protective effects against CIN<sup>147</sup> and cardiac surgery associated AKI<sup>230</sup> are mixed.

#### 1.4.2.10 Intracellular biochemical disruption.

Ischaemia affecting the proximal tubular cells and medullary cells of the thick ascending limb leads to rapid intracellular ATP depletion, due to the high demand for ATP and limited ability to perform glycolysis within these cells, which preferentially use free fatty acid substrate for ATP generation. Limited availability of intracellular ATP leads to dysfunction of the Sodium/Potassium/ATPase ( $\text{Na}^+/\text{K}^+/\text{ATPase}$ ) transporters, which are found on all renal cells and extensively on the basolateral surface of proximal tubular (PT) cells<sup>231</sup> where they are responsible for 70% of cellular oxygen requirements<sup>232</sup>. The loss of this transporter function leads to an influx of  $\text{Na}^+$  ions and water into the cell and an efflux of  $\text{K}^+$  ions. As a result progressive cellular swelling and intracellular dysfunction occurs and if uncorrected results in cellular membrane rupture and necrotic cell death<sup>233</sup>.

In response to high intracellular  $\text{Na}^+$  levels the Sodium/Calcium ( $\text{Na}^+/\text{Ca}^{2+}$ ) exchange protein, found particularly on the cortical PT basolateral membranes, reverses its usual function and causes significant influx of  $\text{Ca}^{2+}$ . Dysfunction of sarcolemmal ATPase, which is responsible for active transport of  $\text{Ca}^{2+}$  into the endoplasmic reticulum for storage, results in further disruption of intracellular calcium homeostasis. High intracellular  $\text{Ca}^{2+}$  leads to dysregulation of the mitochondrial permeability transition pore (MPTP)<sup>234</sup>, as well as activation of a number of proteases, phospholipases and endonucleases that are responsible for necrotic and apoptotic cell death.

#### 1.4.2.11 Mitochondrial dysfunction

A cascade of intracellular events, including oxidant stress during reperfusion and disruption in intracellular  $\text{Ca}^{2+}$  homeostasis, leads to opening of the mitochondrial permeability transition pore (MPTP)<sup>235</sup>. As a result mitochondria are no longer able to maintain a transmembrane potential gradient of  $\text{H}^+$  ions, critical for oxidative phosphorylation and ATP production. Efflux of  $\text{H}^+$  from the mitochondria is accompanied by increased permeability to intracellular ions, proteins and water which causes swelling of the mitochondria<sup>236</sup> and release of pro-apoptotic molecules into the cytosol. Fission of mitochondria also occurs which is thought to contribute to increasing outer membrane permeability and is a precursor to cellular apoptosis and necrosis in renal IRI<sup>237</sup>. Cyclosporine A is a known indirect inhibitor of MPTP opening and is cardio-protective against IRI<sup>238</sup>, however its use in renal IRI is limited by its nephrotoxic and vasoconstrictive properties<sup>239</sup>.

#### 1.4.2.12 Apoptosis, necrosis and autophagy

Apoptosis describes an orderly and active process of programmed cell self-destruction that occurs in response to severe cellular injury and which manifests during the maintenance phase of ATN. As a number of the enzymes involved in apoptosis require ATP for their function, it can only occur in cells following reperfusion and in those that have recovered their respiratory function to some degree. The hallmark features of apoptosis are initial activation of the 'intrinsic' and 'extrinsic' caspase pathways, which in turn activate nuclease and endonuclease proteins causing nuclear and DNA degradation<sup>225</sup>, formation of apoptotic bodies and cell death. Reduction in the levels of intracellular GTP has also been shown to up-regulate a protein known as p53 which exerts a strong apoptotic effect on the cell<sup>240</sup> (Figure 4).

The cellular membranes of apoptotic bodies remain intact until phagocytosed by macrophages or other leukocytes within the renal parenchyma, which prevents the extrusion of intracellular contents into the interstitium and the corresponding inflammatory response<sup>225</sup>.

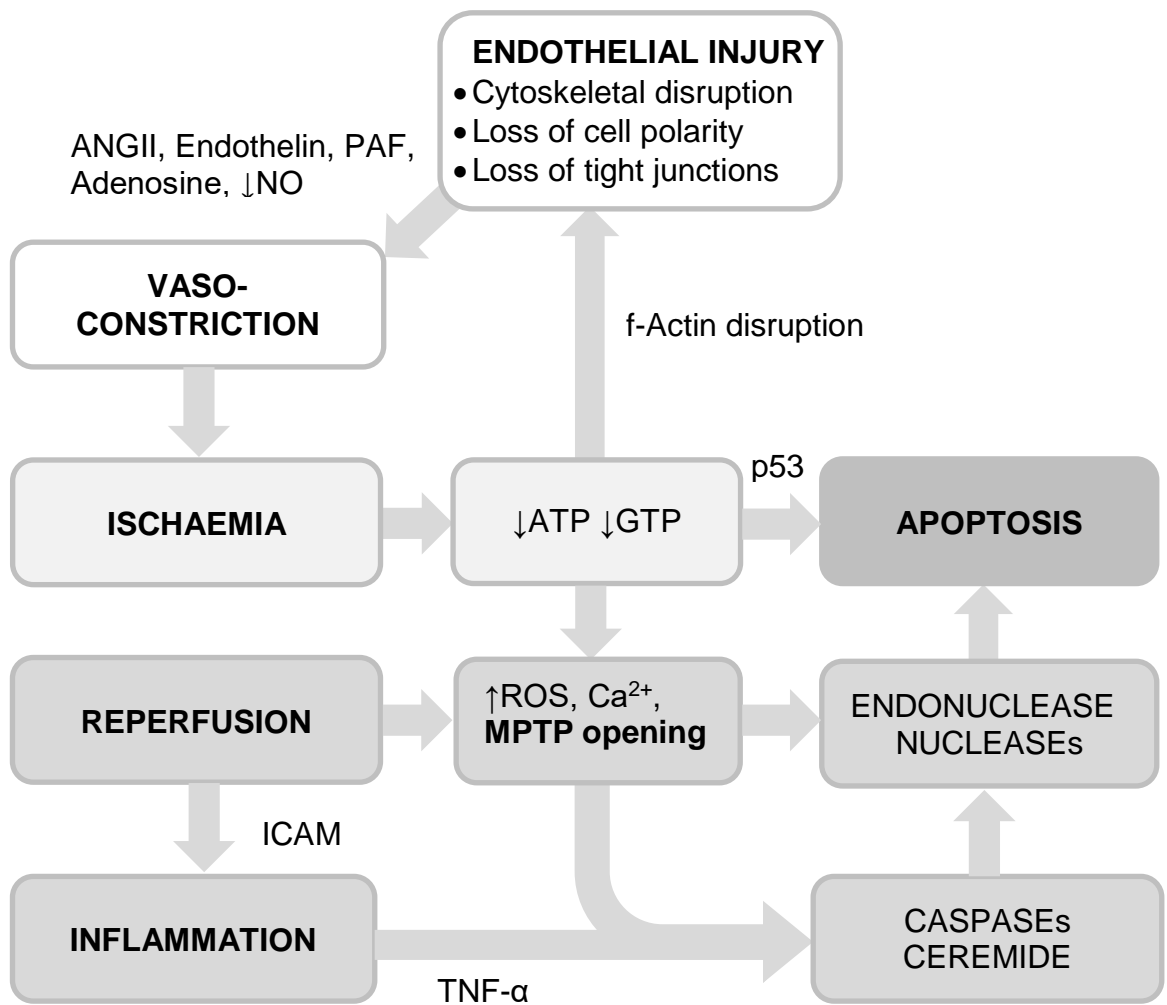
The intrinsic caspase pathway is activated by intracellular ROS, hydrolysis of the phospholipid membrane and generation of ceramide<sup>241</sup>, structural abnormalities in the ATP dependent f-actin cytoskeleton and release of pro-apoptotic proteins such as cytochrome-c from the mitochondria in response to increased permeability across the mitochondrial membrane<sup>242</sup>. The extrinsic caspase pathway is directly activated by a family of TNF-receptor cell membrane proteins, otherwise known as 'death receptors', in response to TNF- $\alpha$  secreted by activated leukocytes<sup>225</sup>.

Cells that are not re-perfused or those that are critically injured to a degree where ATP production in sufficient quantities is impossible, undergo disorderly necrosis with passive swelling and rupture of cellular organelles and the cell membrane. This is followed by extrusion of cellular contents into the extracellular matrix, exacerbating local tissue injury through activation of inflammatory pathways<sup>225</sup>. Both apoptosis and necrosis occur simultaneously in IRI with differentiation between the two types of cell death largely attributable to intracellular ATP concentration following reperfusion.



Autophagy is a catabolic determinant of cell viability that involves orderly degradation of damaged cellular organelles and proteins by proteolytic lysosomal enzymes. Ischaemia induces autophagy following reduced oxidative phosphorylation of ATP, with resulting increase in AMP, AMP kinase and inhibition of mTOR activity<sup>243</sup>. During reperfusion autophagic mediators include ROS<sup>244</sup>, Beclin-1, phosphatidylinositol 3-kinase (PI3K)<sup>245</sup>, AKT<sup>246</sup>, and heat shock proteins (HSP)<sup>247</sup>. Autophagy may be protective in renal IRI<sup>248</sup> when associated with telomerase reverse transcriptase (TerT) activation as TerT deficient mice have been shown to suffer aggravated renal IRI with a reduction in cellular autophagy<sup>249</sup>. This may partially explain the increased susceptibility to AKI in the elderly population who have intrinsically lower TerT activity and shorter telomere length. However autophagy has been shown to be detrimental when mediated by activation of Beclin-1<sup>250</sup>, inhibition of which can be mediated via IPC activation of the RISK pathway. Activation of autophagy prior to IRI is thought to be protective, whereas inhibition of autophagy during reperfusion may be beneficial<sup>251</sup>.

Figure 4: Apoptotic pathway in ischaemia-reperfusion injury of the kidney



Abbreviations: ANGII = Angiotensin II, PAF = Platelet activated factor, NO = Nitric oxide, ATP = Adenosine triphosphate, GTP = Guanosine triphosphate, ROS = Reactive oxygen species, MPTP = Mitochondrial permeability transition pore, TNF = Tissue Necrosis Factor

## 1.5 ORGAN PROTECTION AND ISCHAEMIC CONDITIONING

The concept of organ protection against IRI was first proposed in 1986 by Murry, Jennings and Reimer<sup>252</sup>, who described therapeutic attenuation of the extent of myocardial infarction in a canine model, beyond that achieved by timely reperfusion alone. This seminal study demonstrated that ‘preconditioning’ the heart with transient ischaemia, achieved via intermittent ligation of a coronary vessel (four 5 minute cycles of sub-lethal ischaemia followed by reperfusion), protected the distal myocardium from a subsequent lethal IRI induced by a 40 minute coronary ligation. A dramatic infarct size reduction of 75% was observed which confirmed that reperfusion injury was both an observable entity and a modifiable process.

### 1.5.1 ISCHAEMIC PRECONDITIONING

This phenomenon was termed ‘classical’ ischaemic preconditioning (IPC) and is the result of direct activation of an endogenous protective response within a target organ, the mechanisms of which will be discussed in this chapter. Classical IPC appears to be a ubiquitous process that has been reproducibly demonstrated in a wide range of animal models and in humans and is capable of conveying protection against IRI in the heart as well as in other organs including the kidney<sup>253</sup>, brain<sup>254</sup>, intestine<sup>255</sup>, liver<sup>256</sup>, lung<sup>257</sup>, skeletal muscle<sup>258</sup> and skin<sup>259</sup>. However the invasive nature of IPC and its restriction to predictable models of ischaemia make clinical application limited. The interest generated by IPC nevertheless led to investigation of many other forms of conditioning including ‘remote’ ischaemic preconditioning (RIPC) applied to a distant organ prior to ischaemia, remote per-conditioning (RIPerC) applied to a distant organ during ischaemia, ischaemic post-conditioning (IPostC) applied after ischaemia, remote ischaemic post-conditioning (RIPostC) applied to a distant organ after ischaemia and pharmacological conditioning.

### **1.5.1.1 Temporal aspects of ischaemic conditioning**

The main clinical limitation of classical IC (and RIPC) is that onset of injurious ischaemia in important and potentially amenable conditions (e.g. ACS, CVA and ischaemic AKI) is usually unpredictable, rendering pre-treatment impossible. However there may be a role for preconditioning in clinical scenarios where IRI is predictable (e.g. myocardial injury during elective CABG surgery and CIN in coronary angiography). Nevertheless pre-treatment does not appear to be an absolute requirement for organ protection, as benefit has been documented during concurrent ischaemia (per-conditioning) and after ischaemia conditioning (post-conditioning)<sup>260</sup> (Figure 5).

### **1.5.1.2 Biphasic response to ischaemic preconditioning**

The protective response against IRI following classical IPC and RIPC is effective in target tissues almost immediately following the IC stimulus and persists for up to 4 hours, after which time it declines rapidly<sup>261</sup>. Following this, a ‘second window of protection’ (SWOP), otherwise known as ‘late’ or ‘delayed’ preconditioning, occurs at 24 hours and persists for up to three days although its effect is of lower magnitude<sup>262</sup> (Figure 6). The second component of this biphasic response is not fully understood however it is thought to be mediated via up-regulation of various proteins including iNOS, COX-2 and heat shock proteins (HSP’s) within the target organ tissue. HSP’s are believed to have several functions including stabilisation of the intracellular cytoskeleton, protein chaperoning (assisting with assembly or repair of newly formed or damaged proteins) and down-regulating non-essential protein synthesis in damaged cells<sup>263</sup>. These temporal considerations have allowed IC to exist as a potential treatment strategy for a wide range of predictable and unpredictable IRI conditions however it is critical to recognise the importance and limitations of the proven windows of efficacy.

Figure 5: Temporal considerations in ischemic conditioning

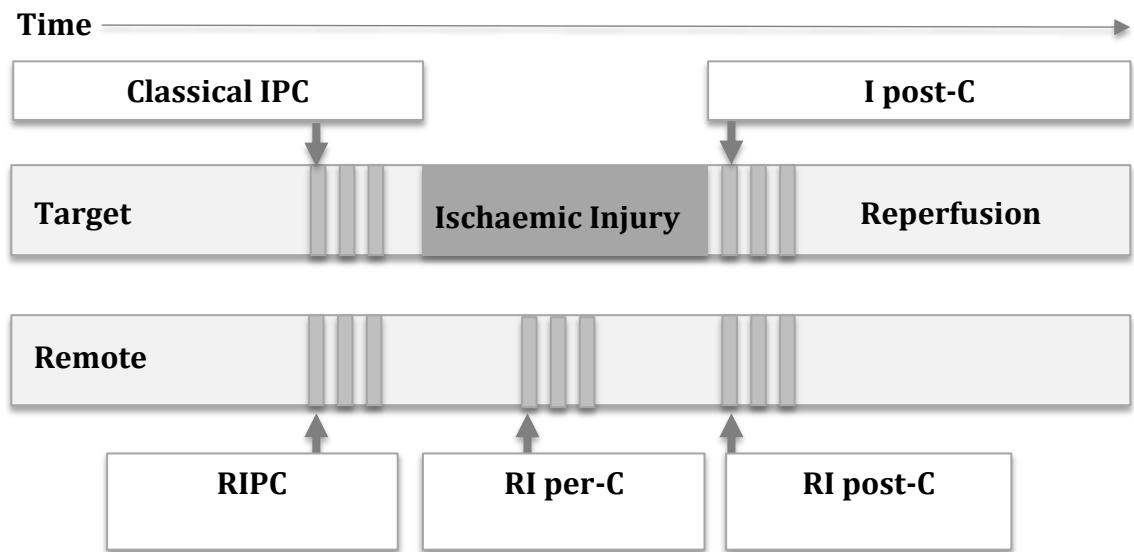
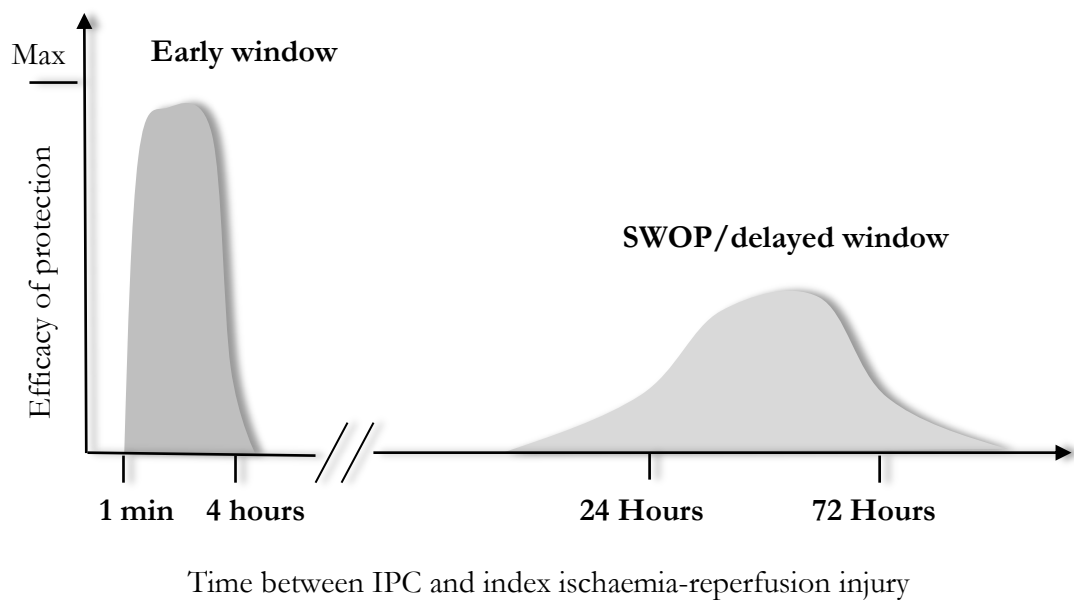


Figure 6: Biphasic windows of efficacy following ischaemic preconditioning



## 1.5.2 PROPOSED MECHANISM OF ISCHAEMIC PRECONDITIONING

The resistance to IRI that occurs following an IPC stimulus is mediated through ischaemia related ligand activation of cell surface receptor ‘trigger mechanisms’ as well as direct RISK pathway activation secondary stimuli such as intracellular acidosis<sup>264</sup>. Once activated, the trigger mechanism initiates a complex signalling cascade within the cell known as the ‘signal transduction pathway’ which terminates at an ‘end effector’, ultimately responsible for determining cell survival (Figure 7). In addition a ‘memory’ component, likely mediated through protein kinase C and post translational modification of intracellular proteins, is believed to be responsible for the windows of efficacy seen in early (0-4 hours) and delayed (24-76 hours) IPC respectively<sup>265</sup>. Much of the basic research into this area involves myocardial cell pathways, which in general exhibit commonality with other cell types such as in the kidney, however where renal pathways have been determined these will be elucidated.

### 1.5.2.1 Trigger mechanism

Numerous cell surface receptors have been identified as playing a role in the trigger mechanism of IC, which can be broadly grouped into G<sub>i</sub> Protein-coupled receptors (GPCR), growth factor (GF) receptors and ‘other’ ligand receptors<sup>266</sup>. These receptors known as ‘preconditioning mimetics’, are believed to trigger an IRI resistant phenotype through summative activation beyond a threshold for response<sup>267</sup>. Ischaemic activation of GPCR and GF receptors by their respective ligands, results in activation of receptor associated tyrosine-kinase, which in turn activates signalling cascades, including the RISK pathway. In addition, non-receptor triggering of the RISK pathway by pharmacological agents (section 1.5.3.6), as well as direct RISK activation secondary to intracellular acidosis and other metabolic imbalances are thought to occur<sup>264</sup>.

In the heart and kidney the GPCR ligand/receptor complexes potentially implicated in IC include adenosine  $A_{2b}$ <sup>268</sup>, opioid<sup>269</sup>, glucagon-like peptide 1 (GLP-1)<sup>270</sup> and adrenomedullin<sup>271</sup>. Interestingly there is no evidence for renal involvement of the cardio-protective autacoids urocortin<sup>272</sup> and bradykinin<sup>273</sup>, with the latter potentially aggravating renal IRI<sup>274</sup>. GF ligand/receptors with both cardio and reno-protective effects include insulin<sup>275</sup>, transforming growth factor beta (TGF- $\beta$ )<sup>276</sup>, granulocyte colony stimulating factor (G-CSF)<sup>277</sup>, fibroblast growth factor 2 (FGF-2)<sup>278</sup>, erythropoietin<sup>279</sup> and the adiponectin leptin<sup>280,281</sup>. Further cardio-protective GF ligand/receptors with as yet unproven renal effects include insulin-like growth factor-1 (IGF-1)<sup>282</sup>, corticotrophin-1 (CT-1)<sup>272</sup> and other adiponectins including visfatin<sup>283</sup>, apelin<sup>284</sup>, although not resistin<sup>285</sup>. Other ligand/receptors include atrial natriuretic peptide<sup>286</sup> and oestrogen<sup>287</sup>. Many of these receptor/ligand entities are suitable targets for pharmacological intervention, with appropriate exogenous drug ligands capable of triggering a protective response (section 1.3.8).

### 1.5.2.2 Signal Transduction pathways

Cell surface receptor triggering and parallel signal transduction leads to the activation of a number of intracellular protein kinases, e.g. protein kinase-C (PKC)<sup>288</sup>. Following GPCR or GF receptor activation, a complex signalling pathway is initiated via phosphatidylinositol 3-kinase (PI3-K) activated AKT (protein kinase B), which in turn activates endothelial nitric oxide synthase (eNOS). The resulting nitric oxide (NO) production, which may be supplemented by EPO mediated activation of Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signalling pathway, activates guanylate cyclase (GC) which activates cyclic GMP dependant protein kinase (PKG). This pathway results in opening of  $mK_{ATP}$  channels with subsequent mitochondrial  $K^+$  influx. During reperfusion this leads to production of mitochondrial ROS which activates PKC<sup>288</sup> and inhibits MPTP opening.

This particular pathway may not play an important role in the kidney, as studies have shown that  $mK_{ATP}$  channel opening is not necessary for RISK pathway activation, which instead relies on direct PI3K/AKT and  $A_{2b}$  receptor/PKC activation<sup>289</sup>. In cardio-myocytes, adenosine is capable of activating PKC directly via the  $A_1$  and  $A_3$  receptor, however the role of these receptors in the kidney is less clear. Animal models have confirmed the renoprotective effect of specific  $A_1$  agonists<sup>290</sup>, however specific  $A_3$  agonists have been shown to be deleterious to AKI<sup>253</sup>. Activation of PKC is thought to be a key step responsible for the ‘memory’ effect that creates the early window effect following the initial trigger. In both organs, activated PKC potentiates low affinity  $A_{2b}$  receptor activation of both AKT and ERK 1/2 in the RISK pathway, which in turn leads to inhibition of mitochondrial permeability transition pore (MPTP) opening via glycogen synthase kinase-3beta (GSK-3 $\beta$ ), ultimately protecting the cell against apoptotic cell death<sup>291</sup>. In cardio-myocytes, IPC mediated RISK pathway activation via PI3K/AKT also leads to inhibition of Beclin-1<sup>292</sup>, which reduces cellular autophagy and appears to prevent cell death (section 1.4.2.12). In vitro and animal models also suggest Beclin-1 also plays a role in exacerbating renal IRI<sup>293</sup> and is likely to be amenable to IPC via the same pathway.

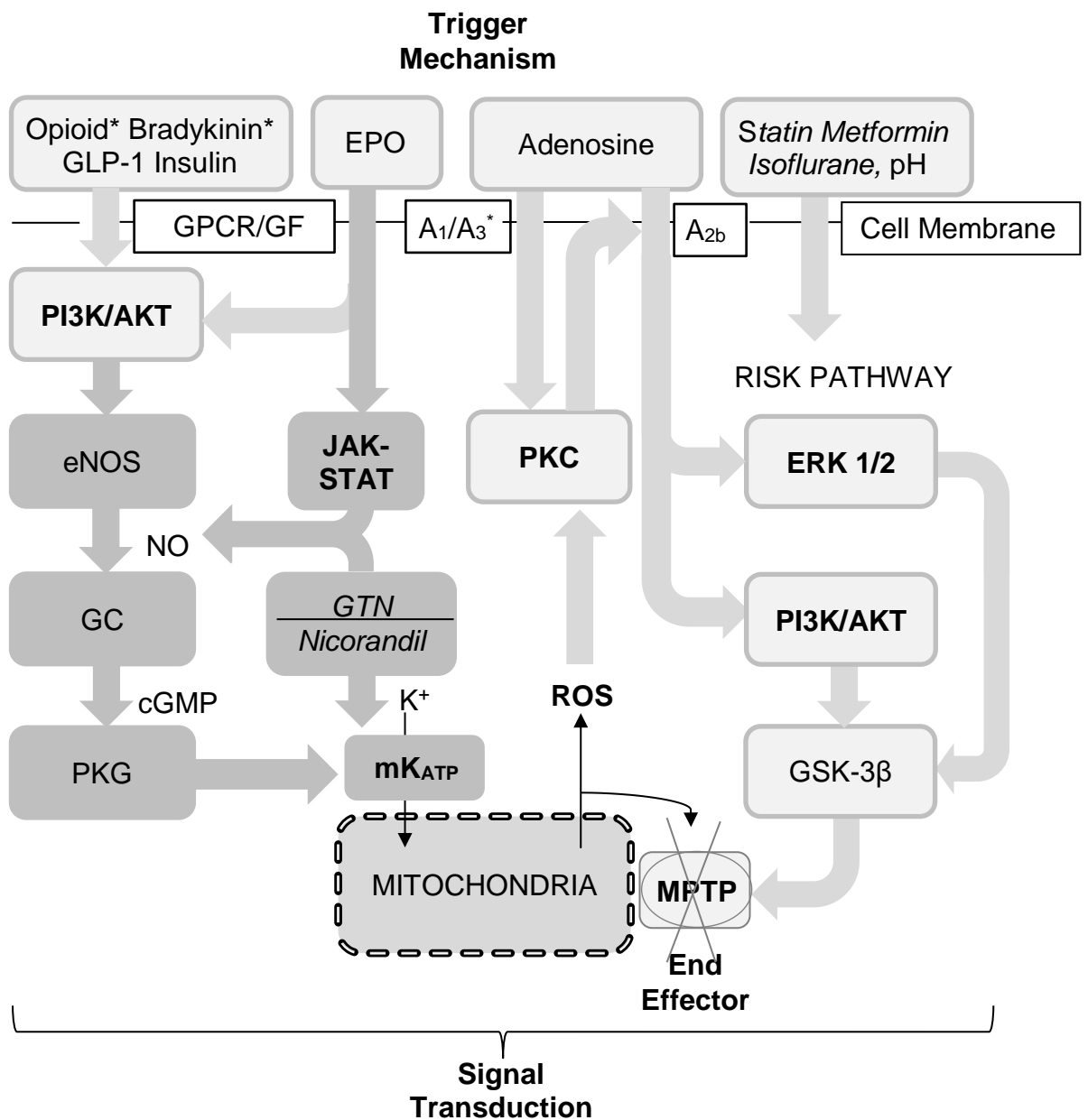


### 1.5.2.3 End effector

The principle target that the signal transduction pathways converge upon is believed to be the mitochondrial transition pore (MPTP), the constituents of which remain under investigation, however opening of the MPTP ante-cedes cell apoptosis and inhibition of opening conveys a resistant phenotype<sup>294</sup>. One component of this pore is formed from an adenine nucleotide translocator (ANT) protein on the inner mitochondrial membrane and a voltage-dependent anion channel on the outer membrane, which when aligned connect the matrix directly with the cytosol. This permits efflux of H<sup>+</sup> ions from the matrix resulting in loss of the membrane potential and inhibition of oxidative phosphorylation, critical for cell survival. In addition efflux of pro-apoptotic mediators such as cytochrome C is thought to occur (section 1.4.2.12).

An important component of cellular autophagy known as mitophagy, involves depolarisation of mitochondrial membranes and fragmentation of mitochondrial complexes (fission) in order to allow degradation of damaged mitochondria. Ischaemic mitochondrial fragmentation is believed to be mediated by mitochondrial fission protein (DRP1)<sup>295</sup> and mitophagy is then regulated by translocation of cytosolic ubiquitin ligase (parkin) to the mitochondrial membrane where it fuses with mitofusin 2 which is then phosphorylated by phosphatase and tensin homologue (PTEN) induced kinase 1<sup>296</sup>. Mitophagy has been shown to be cardio-protective against IRI in experimental studies mediated through removal of potentially harmful defective mitochondria<sup>297</sup>. However prevention of mitochondrial fragmentation by direct inhibition of DRP1<sup>295</sup> and mitofusin 2<sup>298</sup> has also been shown to prevent MPTP opening and convey cardio-protection, which suggests a wider role of these proteins beyond that of mitochondrial fission alone, although it is not known at present whether IPC influences these pathways<sup>299</sup>.

Figure 7: Preconditioning mechanism



➡ = Cardiac & renal protection pathway    ➡ = Cardiac protection pathway only

\*= Unknown or deleterious effect in renal IPC, *italics* = pharmacological conditioning

Abbreviations: GLP-1 = glucagon like peptide-1, EPO = erythropoietin, GPCR = G Protein coupled receptor, GF = growth factor receptor, A<sub>1</sub>, A<sub>2b</sub>, A<sub>3</sub> = adenosine A<sub>1</sub>, A<sub>2b</sub>, A<sub>3</sub> receptors, Met = Metformin, PI3-K = phosphatidylinositol 3-kinase, eNOS = endothelial nitric oxide synthase, NO = nitric oxide, EPO = erythropoietin, JAC-STAT = Janus-activated kinase signal transducer and activation of transcription, GTN = glyceryl tri-nitrate, GC = guanylate cyclase, cGMP = cyclic Guanisine monophosphate, PKG = protein kinase G, mK<sub>ATP</sub> = mitochondrial potassium-adenosine triphosphate channel, ROS = reactive oxygen species, PKC = protein kinase C, ERK = extracellular signal-regulated protein kinase, GSK-3b = glycogen synthase kinase-3b, mPTP = mitochondrial permeability transition pore. Adapted from Downey et al<sup>300</sup> & Sivaraman et al<sup>301</sup>

### 1.5.3 PHARMACOLOGICAL CONDITIONING

The various cell surface receptors, signal transduction pathways and end effectors in IPC offer ideal pharmacological targets that are capable of potentiating a similar protective response against IRI.

#### 1.5.3.1 Adenosine

Exogenous administration of adenosine is an obvious choice given its interactions with  $A_1$ ,  $A_{2b}$  and  $A_3$  receptors in the IPC trigger mechanism and its  $A_{2a}$  mediated anti-inflammatory properties. Despite encouraging data in animal models of myocardial IRI<sup>302</sup>, results in clinical studies have been mixed with the Amistad I<sup>303</sup> and II<sup>304</sup> STEMI trials demonstrating reduced infarct sizes but no reduction in MACE, whilst other similar PPCI trials have not shown benefit<sup>305</sup>. This variability might be explained by adenosine's short in-vivo half-life (<10 seconds) and lack of specificity, however whilst specific adenosine receptor agonists have shown promise in animal models<sup>306</sup>, translation into benefit in clinical trials has not yet been determined.

The ADMIRE study<sup>307</sup> which used a novel  $A_1/A_2$  receptor agonist during PPCI, was only able to demonstrate a non-significant trend towards reduction in infarct size. The role of adenosine within the kidney is complex (section 1.4.2.4). Adenosine antagonists, such as aminophylline and theophylline, have shown equivocal reno-protective effects in CIN clinical trials<sup>168</sup>, with their vasodilatory benefits possibly attenuated by inhibition of preconditioning pathways. The sedative dexmedetomidine, a specific  $A_{2a}$  agonist, and has demonstrated reno-protection in a rat model of AKI, thought to be mediated via modulation of autophagy via MAPK p38, as well as through a preconditioning effect mediated via PI3/AKT<sup>308</sup>.

Other specific  $A_1$ <sup>309</sup> and  $A_{2a}$ <sup>211</sup> receptor agonists have also been shown confer reno-protection through anti-inflammatory effects in experimental studies. A retrospective analysis of patients receiving dexmedetomidine during CABG surgery has suggested improved cardiovascular outcomes<sup>310</sup> and a lower incidence of AKI<sup>311</sup>, however translation into prospective clinical studies has yet to occur.

### **1.5.3.2 Opioids**

Exogenous opioids such as Morphine have been shown to be both cardio and reno-protective<sup>312</sup> in animal models, likely through activation of the delta and kappa opioid and EGF receptors with transduction via the PI3K/AKT and the mK-ATP pathway (Figure 7). Opiates are commonly used during many clinical IRI scenarios (e.g. CABG and STEMI), however there is only limited evidence for their efficacy in IRI in clinical trials<sup>313</sup>.

### **1.5.3.3 Other exogenous autacoids**

Atrial natriuretic peptide (ANP) is a hormone produced by the cardiac atria which acts on the kidney to increase renal medullary blood flow and glomerular filtration rate. It has been shown to be cardio<sup>314</sup> and reno-protective<sup>315</sup> against IRI via activation of guanylate cyclase (GC) and increased intracellular cGMP<sup>316</sup>. A pilot study by Sward et al<sup>317</sup> investigated i.v. ANP following perioperative AKI in complex cardiac surgery and Mitaka et al<sup>318</sup> investigated i.v. ANP prior to aortic cross-clamping in abdominal aortic aneurysm (AAA) repair, both demonstrating attenuation of AKI, although both studies were unable to distinguish whether improved renal haemo-dynamics or a direct protection was responsible.

The functional GLP-1 analogue 'Exenatide', used in the treatment of diabetes mellitus, has shown promising cardio-protective properties in experimental models and proof of concept clinical trials<sup>319, 320</sup>, however it has not been investigated in the setting of AKI. Nevertheless, Sitagliptin, which up-regulates production of GLP-1 and receptors has recently been shown to protect against AKI in a rat model<sup>321</sup>.

Other exogenous autacoid ligands with evidence against renal IRI in animal models include Insulin<sup>275</sup>, GCSF<sup>277</sup> and Erythropoietin<sup>322</sup>. Insulin therapy has been shown to be of benefit in protecting against AKI in a systematic review of critically ill patients<sup>323</sup>, however the clinical efficacy of GCSF is untested. Erythropoietin has not displayed clinical benefit in cardio-protection and in fact may be harmful<sup>324</sup> whilst efficacy in preventing ischaemic AKI remains equivocal<sup>325</sup>.

#### 1.5.3.4 Nitrates

GTN is an anti-anginal vasodilatory medication which is thought to exert its protective effect through NO donation and activation of guanylyl cyclase which promotes  $mK_{ATP}$  opening via increased cGMP levels. A historical meta-analysis investigating GTN use during ACS demonstrated significant mortality benefits<sup>326</sup>. In addition, Candilio et al<sup>327</sup> recently reported in a subgroup analysis that perioperative use of GTN in CABG significantly reduced the incidence perioperative MI (PMI) by 38%. They also demonstrated that GTN and RIPC were equally effective in preventing PMI, but did not confer additive benefit when used in combination, suggesting that both therapies may operate through a common pathway. Experimental evidence in a rat model<sup>328</sup> also supports the reno-protective role of GTN when administered prior to ischaemic AKI, however no effect was seen when administered following renal IRI. No clinical studies have specifically investigated this effect and Candilio et al have yet to report on the effect of GTN on AKI in subgroup analysis.

#### 1.5.3.5 Other $K_{ATP}$ agonists

Nicorandil, another antianginal drug which is a direct  $K_{ATP}$  channel agonist as well as a NO donor with equivocal cardio-protective effects in STEMI<sup>329, 330</sup>, has shown promise in animal models of ischaemic AKI<sup>331</sup> however has not been effective in ameliorating CIN in clinical trials<sup>332</sup>. Levosimendan, a calcium sensitising heart failure medication that also has  $K_{ATP}$  agonist effects, has demonstrated reno-protection against IRI in a porcine model<sup>333</sup> but has yet to be tested in clinical studies.

### 1.5.3.6 Pharmacological targets within the RISK pathway

Volatile anaesthetics such as isoflurane<sup>334</sup> have demonstrated both cardio and reno-protection, thought to be mediated by sphingosine 1-phosphate receptor activation of AKT/ERK. One facet of the pleiotropic cardio-protective effects of Statins may be attributable to direct activation of the RISK pathway as demonstrated by Bell et al<sup>335</sup>. As described (section 1.3.8.2), statins have been shown to protect against CIN in vitro and in clinical studies<sup>336</sup>. Metformin has also been shown to mediate cardio-protection through activation of the RISK pathway<sup>337</sup>, inhibition of pro-apoptotic caspase pathways and upregulation of mitogen activated protein kinases (mAPK) p38. No clinical trials have examined the benefit of metformin in ischaemic AKI, almost certainly due to the risk of precipitating lactic acidosis.

### 1.5.3.7 Pharmacological targets within mitochondria

Cyclosporin-A is known to directly inhibit MPTP opening and small clinical trials have shown cardio-protective effects<sup>338</sup>, with larger RCT's currently underway<sup>339</sup>. However its use is limited by nephrotoxicity and as such no specific clinical trials have performed in renal IRI. Trimetazidine, an anti-anginal medication that inhibits fatty acid oxidation and promotes efficient glucose oxidation in ischaemic tissue, is known to preserve mitochondrial homeostasis. Clinical trials have demonstrated both cardio-protection during PCI<sup>340</sup> and reno-protection against CIN<sup>341</sup>. Diazoxide, an antihypertensive with  $K_{ATP}$  channel activating properties, was traditionally assumed to exert a cardio-protective effect through mK-ATP channel activation, however Minners et al found instead that it may be due to mitochondrial respiratory inhibition triggered ROS signalling<sup>342</sup>. Novel mitochondrial antioxidant drugs such as MitoQ, that aim to reduce mitochondrial injury due to excessive ROS production, are currently showing promise in animal models of ischaemic AKI<sup>343</sup>.

## 1.6 REMOTE ISCHAEMIC PRECONDITIONING

The invasive limitations inherent in classical IC were circumvented following the discovery in animal models that brief cycles of non-lethal ischaemia and reperfusion in one organ or tissue induces protection against subsequent lethal IRI in a distant organ or tissue, a phenomenon known as ‘remote’ ischaemic preconditioning (RIPC). In 1993 Whittaker and Pryzlenk<sup>344</sup> demonstrated in a canine model that intermittent occlusion of the left circumflex (LCx) coronary artery protected against subsequent lethal IRI in the neighbouring left anterior descending (LAD) coronary artery territory. This discovery led to a mathematical model<sup>345</sup> that deduced that RIPC had activated, produced, or transported via diffusion, a ‘protective substance’ within the target organ or tissue, although it was unknown what this substance might be. The detection of a communicable intra-organ protective signal led investigators to postulate that inter-organ protection might also be possible, communicated by either humoral, neural or systemic response pathways.

The first evidence of inter-organ protection was established by McClanahan et al<sup>346</sup> in a rabbit model, where occlusion of a renal artery for 10 minutes reduced the extent of a subsequent myocardial IRI, importantly to the same degree as direct myocardial IPC. Confirming these findings, Gho et al<sup>347</sup> utilised 15 minutes of non-injurious mesenteric ischaemia to protect against subsequent myocardial IRI in a rat model, again producing similar infarct size reduction as 15 minutes of direct myocardial IPC. Additionally this effect appeared to be dependent on reperfusion of the mesentery, and that a neuronal pathway was involved in relaying a protective signal to the heart, as the protective effect was abolished when hexamethonium, a ganglion blocker, was administered. RIPC has since demonstrated clinical efficacy in a number of settings utilising both limb ischaemia and inter-organ protection as summarised in Figure 8 and Table 14.



### 1.6.1 RIPC AND LIMB ISCHAEMIA

Transient ischaemia of an upper or lower limb has the advantage of being easily applied and well tolerated, as limb tissues are relatively resistant to IRI. Intermittent occlusion of the vascular supply of a limb is either achieved invasively, e.g. by infra-renal aortic or iliac artery cross-clamping, or more commonly by non-invasive methods such as using a tourniquet, or more reliably and comfortably by inflating a blood pressure cuff to a supra-systolic pressure.

In 1997 Birnbaum et al<sup>348</sup> provided initial experimental data confirming skeletal muscle RIPC cardio-protection in a rabbit model, using electrical stimulation of a hind-limb muscle and 30 minutes of partial occlusion of a femoral artery to induce ischaemia whilst allowing for transport of a hypothesised humoral signal out of the ischaemic zone. As such they demonstrated a 65% reduction in myocardial infarct size from a subsequent coronary artery ligation. Weinbrenner et al<sup>349</sup> examined the effects of varying RIPC occlusion times in a rat model using 15 minutes, 10 minutes and 5 minutes of infra-renal aortic occlusion which resulted in proportional reductions in subsequent myocardial infarct size of 65%, 30 % and 20% respectively, which suggested that an optimal RIPC stimulus duration exists. Further findings in this study included confirmation that PKC is involved in the intracellular signalling pathway (as with IPC) and that the connective pathway between the remote and target organs was likely to have a humoral component as hexamethonium neural blockade did not abolish the observed protective effect.

Evidence for skeletal muscle RIPC reno-protection in animal models is scarce, with evidence from clinical studies vastly outweighing the experimental data. Nevertheless Lazaris et al demonstrated in a rat model that infra-renal aortic occlusion for 15 minutes reduced a subsequent renal IRI due to 45 minutes of sub-phrenic aortic cross clamping.

The first experimental evidence for non-invasive RIPC in large animals was developed by Kharbanda et al<sup>350</sup> who demonstrated in a porcine model, that 4 cycles of 5 minute hind-limb ischaemia was capable of reducing subsequent myocardial IRI during cardiopulmonary bypass. In the clinical component of this trial they also demonstrated in healthy volunteers, that upper limb RIPC using three 5 minute cycles of blood pressure cuff inflation to 200mmHg, was able to protect against a subsequent endothelial IRI in the contralateral forearm caused by a 20 minute blood pressure cuff inflation (200mmHg). This method has been adopted as the standard when administering RIPC to patients, and is a well-tolerated and safe and inexpensive intervention. Lower limb RIPC is also efficacious, and has the potential to provoke a larger response given the increased bulk of ischaemic skeletal muscle, however it is more uncomfortable than upper limb RIPC and thus is usually reserved for anaesthetised patients.

### **1.6.2 TEMPORAL CONSIDERATIONS IN RIPC**

Evidence in man for early and delayed windows of efficacy in RIPC, as seen in IPC, was determined by Loukogeorgakis et al<sup>351</sup>, who applied RIPC to one arm of healthy volunteers and were able to measure an endothelial response, known as ‘flow mediated dilatation (FMD)’ in the contralateral arm. This appeared almost immediately following RIPC and persisted for up to four hours with re-emergence for further period between 24 and 48 hours. The role of a neural signalling pathway was also supported as FMD was abolished by autonomic ganglion blockade. Studies utilising organ RIPC often involve continuous periods of ischaemia of up to 15 minutes in duration, whereas limb RIPC utilises a fractionated approach. The optimal number or length of RIPC cycles remains unknown, with most studies empirically using three or four cycles of 5 minutes ischaemia interspersed with 5 minutes of reperfusion. ‘Hyper-conditioning’ which uses longer ischaemic periods or a greater number of cycles is as yet unexplored<sup>352</sup>.

Figure 8: RIC inter-organ protection

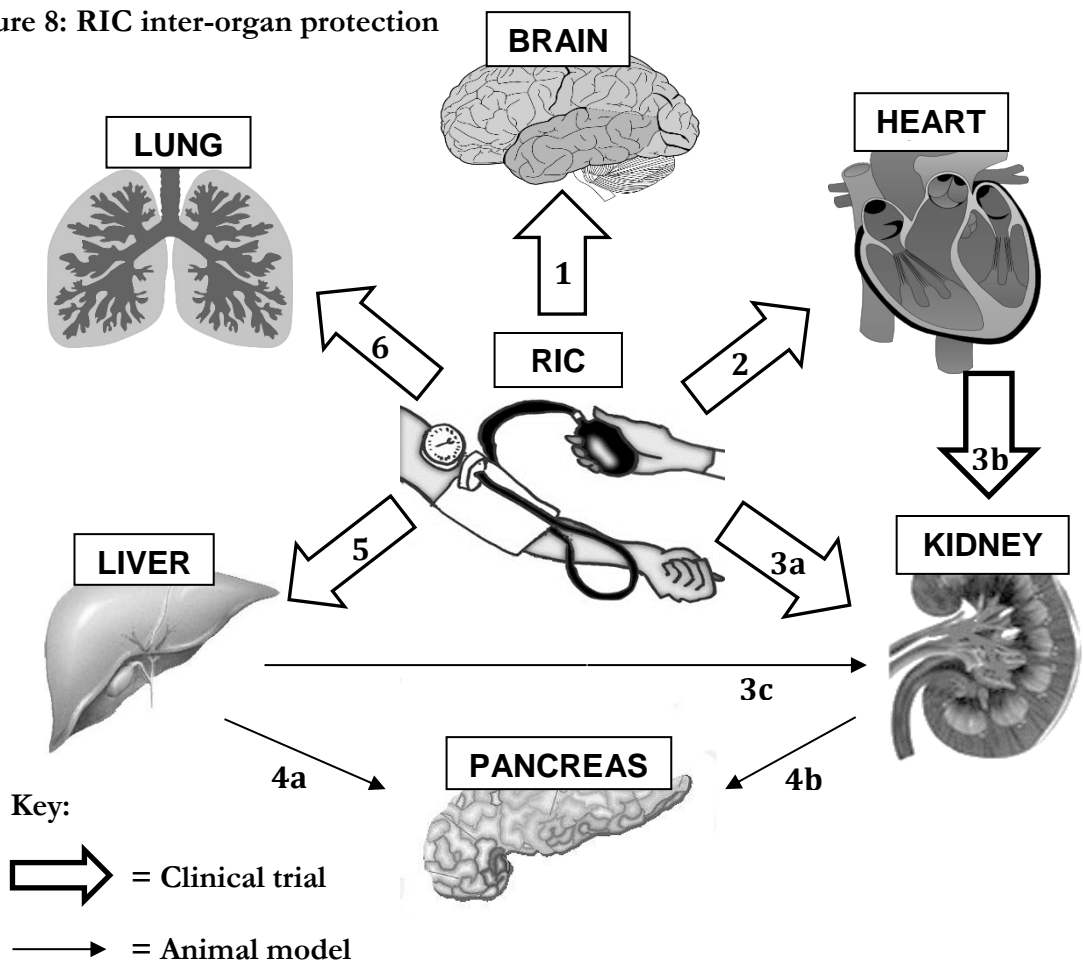


Table 14: Major clinical and experimental studies in RIC organ protection

	Remote IC → target organ	Setting	Size & outcome
<b>1</b>	Upper limb RPerC → Brain	Traumatic brain injury <sup>353</sup>	n= 60, ↓ TBI biomarker
<b>2</b>	Upper limb RPerC → Heart Upper limb RIPC → Heart	STEMI, PPCI <sup>354</sup> CABG <sup>327</sup>	n = 197, ↓MI size n= 180, ↓TnT
<b>3a</b>	Upper limb RIPC → Kidney  Lower Limb RIPostC	PCI, CIN <sup>189</sup> Cardiac surgery, AKI <sup>355</sup> Renal Transplant <sup>356</sup> PPCI, CIN <sup>357</sup>	n= 100, ↓SCr>25% n= 240, ↓SCr>25% n= 406, ↑eGFR n= 97, ↓SCr>25%
<b>3b</b>	Heart RPerC → Kidney	PCI, CIN <sup>191</sup>	n= 225, ↓SCr>25%
<b>3c</b>	Liver RIPC → Kidney	Rat <sup>358</sup>	↑SCr, histopathology
<b>4a</b>	Liver RIPC → Pancreas	Rat <sup>359</sup>	↓ pancreas biomarkers
<b>4b</b>	Renal RIPC → Pancreas	Rat <sup>360</sup>	↑ pancreas microcirculation
<b>5</b>	Lower limb RIPC → Liver	Cardiac surgery <sup>361</sup>	n= 201, ↓ LFT
<b>6</b>	Lower limb RIPC → Lung	Cardiac surgery <sup>361</sup>	n= 201, ↓ Lung biomarkers

Abbreviations: TBI = traumatic brain injury, TnT = troponin T, MACE = major adverse cardiovascular event, SCr = serum creatinine, LFT = liver function tests. Adapted from Hausenloy et al<sup>362</sup> & Candilio et al<sup>363</sup>

### 1.6.3 PROPOSED MECHANISM OF RIPC

Although unified understanding of RIPC is incomplete, several mechanistic pathways are believed to be involved, that can be separated into three interrelated components; the initial remote organ response to RIC, the message relay pathway between remote and target organs and the target organ protective response, thought to be similar to the response observed in IPC and IPostC. Specific pathways involving the kidney are not well understood, however they are assumed to be similar to those known to be involved in signal communication with the heart. Therefore the pathways involved in cardiac relay will be the focus of discussion unless specific renal pathways have been elucidated. Two hypothesised routes for this relay mechanism exist; namely the humoral and neural pathways.

#### 1.6.3.1 Humoral pathway

Two seminal studies by Dickson et al indicated a humoral route for the relay mechanism; the first demonstrated remote cardio-protection in untreated rabbits using blood transfused from those treated with myocardial IPC<sup>364</sup>; the second involved transfusion of coronary venous effluent from classically conditioned rabbit hearts to untreated rabbit hearts<sup>365</sup>. Shimizu et al<sup>366</sup> demonstrated inter-species cardio-protection (human to rabbit) was possible, mediated by a small (<15kDa) hydrophobic molecule and abrogated by opioid receptor blockade with naloxone. A study by Serejo et al<sup>367</sup>, using transfusion of coronary effluent from conditioned rat hearts to untreated rat hearts, also supported these findings and found that the relay molecule was small (3.5-15kDa) and activated PKC in the target organ. Using the same model, Breivik et al<sup>368</sup> postulated a small molecule (<30kDa) activated the RISK pathway (PI3K/AKT) in recipient rats. The first evidence for a humoral pathway in skeletal muscle RIPC was derived by Konstantinov et al<sup>369</sup>, who demonstrated cardio-protection of transplanted (denervated) donor hearts by hind limb RIPC in the recipient porcine model, a process which appeared to be dependent on activation of  $K_{ATP}$  channels.

Numerous candidate molecules within the humoral pathway have been investigated, including NO and nitrite<sup>370</sup> (generated by RIPC induced endothelial shear stress), stromal derived factor-1 $\alpha$ <sup>371</sup> (SDF1-  $\alpha$ ) and micro-ribonucleic acid-144<sup>372</sup> which is transported in blood by liposomal protein complexes and exosomes<sup>373</sup>. It is not known whether these pathways act independently or in concert however it seems likely that multiple humoral signals are involved, which act directly on the target organ, or act upon intermediary neural pathways that connect with the target organ. Accordingly, Lim et al were able to demonstrate that both humoral and neural pathways were involved in cardio-protection in a rat model, as femoral vein occlusion and femoral/sciatic nerve transection independently abrogated the protective effect of hind limb RIPC

### **1.6.3.2 Neural pathway**

Numerous triggers other than RIPC are capable of activating a protective response through direct stimulation of peripheral nociceptive pathways, with evidence for subsequent humoral transmission of this signal to remote organs. Experimental data for this phenomenon exists for 'remote ischemic preconditioning of trauma' (RPCT) using abdominal skin incisions (mediated by bradykinin and neuronal PKC activation)<sup>374</sup>, direct peripheral nerve stimulation<sup>375</sup>, transcutaneous nerve stimulation<sup>376</sup>, electro-acupuncture<sup>377</sup> and chemo-stimulation of sensory C-fibres with capsaicin<sup>375</sup>. In these studies, peripheral nerve transection, or blockade with local anaesthetic drugs, e.g. lidocaine, abrogated the protective effect. This effect is mirrored in a human model, where dialysate from patients with diabetic neuropathy receiving limb RIPC showed attenuated cardio-protection in rabbits<sup>378</sup> compared with dialysate from patients without neuropathy.

A neural signal is also believed to be directly relayed from the conditioned limb to the target organ by somatosensory (as above), autonomic and spinal cord pathways. The evidence for autonomic involvement is derived from experiments utilising autonomic ganglionic blockers (e.g. hexamethonium) to abrogate the effect of RIPC in both animal<sup>347</sup> and human models<sup>351</sup>. In addition both vagal nerve transection and atropine<sup>379</sup> are capable of diminishing the cardio-protective effects of hind-limb RIPC in a rabbit model. Southerland et al<sup>380</sup> demonstrated that electrical spinal cord stimulation is cardio-protective against IRI in a rabbit model, and involves adrenergic cardiac neurons, with the effect abolished by pharmacological  $\alpha$  and  $\beta$  receptor blockade. Hind limb RIPC induced cardio-protection has also been shown to be abrogated by spinal cord transection<sup>374, 379</sup> as well as by intrathecal opioid receptor blockade<sup>381</sup>.

### 1.6.3.3 Post signal relay in the target organ

Although many autacoids and paracrine factors have been shown to initiate IPC, the trigger mechanisms involved in RIPC are less well understood, however the two final pathways are thought to be broadly similar. Specific evidence for triggers involved in RIPC exists for adenosine via  $A_1$  receptors<sup>382</sup>, which also interact with opioid  $\Delta 1$  &  $\kappa$  receptors<sup>383</sup>, bradykinin via  $B_2$  receptors<sup>374</sup>, SDF1- $\alpha$  via chemokine receptor 4<sup>371</sup> and interleukin 10<sup>384</sup> (in delayed RIPC). The mechanisms of RIPC reno-protection are less clear with some studies ruling out activation of adenosine<sup>385</sup>, bradykinin, opioid and muscarinic autonomic receptors<sup>289</sup>, and other studies demonstrating involvement of opioid receptors<sup>269</sup>. The intracellular signal transduction pathways in RIPC are similar to IPC involving activation of AKT and eNOS<sup>384</sup>, PKC<sup>386</sup>,  $K_{ATP}$  channels<sup>387</sup> and the RISK pathway consisting of PI3K and AKT<sup>388</sup>, ERK 1/2<sup>389</sup> and GSK3 $\beta$ <sup>390</sup>. The end effector in IPC, prevention of opening of the MPTP, is also believed to be the final target of RIPC<sup>391</sup> which manifests the pro-survival cell phenotype.

#### 1.6.4 DEMOGRAPHIC AND CLINICAL FACTOR EFFECTS ON RIPC

In 1995 Szilvassy et al<sup>392</sup> described attenuation of the preconditioning response in hyperlipidaemic rabbits. Following this discovery, the traditional cardiovascular risk factors, endemic within patients enrolled into IPC clinical trials, have been shown to abrogate the protective effect of all methods of conditioning, mediated via dysfunction in cytosolic signalling and mitochondrial responses to conditioning stimuli.

Advanced age has been shown to reduce the efficacy of IPC, as summarised in a comprehensive review by Boengler et al<sup>393</sup>, dependent on reduced activity of the AKT/GS3K- $\beta$  pathway<sup>394</sup> and mitochondrial dysfunction from excessive ROS production<sup>395</sup>. The effect of age on RIPC efficacy is less well understood, with some studies suggesting increased efficacy of RIPC in healthy elderly volunteers<sup>396</sup>. Interestingly although in general females appear to be relatively resistant to IRI<sup>397</sup>, elderly female patients appear to be more responsive to RIPC than elderly males<sup>398</sup>, potentially mediated by gender related isoforms of PKC and ERK<sup>399</sup>.

Patients with diabetes mellitus (DM) are at greater risk of cardiovascular and renal complications and are more susceptible to organ IRI<sup>400</sup>. The majority of experimental and clinical studies have shown that DM interferes with the protective RIPC response, due to the effects of overlapping metabolic comorbidities, inhibition of the protective humoral signal in diabetic peripheral neuropathy<sup>378</sup> and dysfunction of AKT/ERK1/2 in the RISK pathway<sup>266</sup> and JAK-STAT within the SAFE pathway<sup>401</sup>. In addition pharmacological agents used to treat DM<sup>402</sup> inhibit the mK<sub>ATP</sub> channel<sup>403</sup> and disrupt AKT signalling<sup>404</sup>. Nevertheless numerous studies have shown DM patients benefit from RIPC in various clinical settings<sup>405</sup> although with conflicting evidence in CIN<sup>406, 407</sup> suggesting a threshold to response may exist.

Hypertension and LVH has been shown in an aged animal model to raise the threshold for IPC of the myocardium and is unaffected by ACE-I co-therapy<sup>408</sup>. Hyperlipidaemia significantly aggravates myocardial IRI and abrogates the effects of RIPC, as summarised in a thorough review by Ferdinandy et al<sup>409</sup> due to oxidative dimerization of PKG<sup>410</sup> as well as inhibition of  $mK_{ATP}$ <sup>411</sup> and matrix metalloprotease (MMP2), a regulator of the extrinsic apoptotic pathway and RISK pathway<sup>412</sup>. A host of other deleterious genetic and cellular responses to hyperlipidaemia exist, including decreased NO and HSP expression and increased oxidative stress that may play a role in abrogating IPC.

Despite a strong correlation with adverse cardio-renal outcomes, kidney dysfunction does not abrogate the protective effects of IPC<sup>413</sup> where RISK and SAFE signalling pathways remain intact.



## **1.6.5 RIPC AND RENO-PROTECTION**

In 1999, Cochrane et al<sup>414</sup> were the first to demonstrate in a rat model that hind-limb RIPC ameliorated subsequent renal IRI. However translation of RIPC reno-protection into clinical settings, such as the prevention of AKI in patients undergoing major cardiac or vascular surgery, renal transplantation and CIN, has yet to be fully established. Many of the clinical studies have been small, single centre investigations that have not been adequately powered to demonstrate reno-protection. In addition renal and clinical endpoints vary widely between studies and patient populations are variably affected by differing co-morbidities and poly-pharmacy.

### **1.6.5.1 Reno-protection during non-cardiac surgery**

Ali et al<sup>415</sup> used intermittent non injurious iliac artery occlusion prior to surgical abdominal aortic aneurysm repair and demonstrated reduced serum creatinine in RIPC versus control patients. Another randomised trial performed by Walsh et al<sup>416</sup> investigated lower-limb cuff RIPC prior to endovascular abdominal aortic aneurysm repair (EVAR) and found a reduced incidence of subclinical acute kidney injury, indicated by a reduction in the urine albumin creatinine ratio (UACR) and urinary retinol-binding protein levels at 24 hours. However contrary to this a further study by Walsh et al<sup>417</sup> did not demonstrate renal protection using intermittent iliac artery occlusion prior to open infra-renal abdominal aortic aneurysm repair; although a larger retinol-binding protein (a novel marker of kidney injury) increase in control versus RIPC patients was found.

### 1.6.5.2 Reno-protection during cardiac surgery

The evidence for perioperative AKI prevention using RIPC prior to CABG surgery is mixed, following Zimmerman et al's<sup>28</sup> initial demonstration of efficacy in a small study using lower limb RIPC. Retrospective analysis of two studies using upper limb RIPC prior to CABG surgery by Venugopal et al<sup>418</sup> found no SCr reduction, although significantly more control patients were in AKIN category 1 (Table 3). A prospective study by Thielmann et al<sup>419</sup> also using upper-limb RIPC prior to CABG, demonstrated lower peak postoperative SCr level in RIPC patients but no difference in eGFR. Two recent studies demonstrated more convincing evidence; In 2015 Zarbock et al published a study examining the effect of RIPC in 240 high risk patients undergoing CABG and found significant reductions in AKI (37.5% vs 52.5%), renal replacement therapy (5.8% vs 15.8%) and ICU stay (3 days vs 4days). Candilio<sup>327</sup> et al also demonstrated a similar reduction in perioperative AKI (10% vs 21%) in 180 patients undergoing CABG. However this evidence is countered by two recent multicentre randomised controlled trials 'ERICCA<sup>420</sup>' and 'RIP-Heart<sup>421</sup>' which both examined whether RIPC could ameliorate complications resulting from CABG surgery, with no differences found in the secondary outcome measure of perioperative AKI between RIPC and control groups in either study (ERICCA n=1612, 38% vs 38.3%, p=0.975, RIP-Heart n = 1403, 6.1% vs 5.1%, p=0.45).

Although initial pilot studies suggested that RIPC may have a role in ameliorating perioperative AKI during cardiac surgery, the neutral secondary outcomes in large clinical trials suggest that either RIPC may not be an effective intervention in this setting, perhaps due to the complex ischaemic and non-ischaemic injurious processes that occur during cardiac surgery, or that significant confounding factors may exist, for example abrogation of the preconditioning effect by anaesthetic or cardiovascular medications.

### 1.6.5.3 Reno-protection during kidney transplantation

Macallister et al, in the recent multinational REPAIR trial<sup>356</sup>, evaluated RIPC prior to living donor renal transplantation in 406 donor-recipient pairs. Both short and long term windows of efficacy were examined and both donor and recipient received RIPC. Although the primary endpoint of Iohexol GFR measurement showed a non-significant trend towards benefit in early RIPC, the secondary measurement of eGFR as calculated by SCr at six months demonstrated significant improvement in early RIPC (adjusted difference 4.98, 95% CI 1.13 to 8.29;  $p = 0.011$ ). Late RIPC however was not associated with any reno-protective benefit. As such this important study demonstrated that RIPC may have an important role to play in renal as well as other organ transplantation by extending the duration of donor organ survival through minimisation of ischemic organ damage during transplantation.

#### 1.6.5.4 CIN prophylaxis during coronary angiography and PCI

Er et al<sup>189</sup> were the first to convincingly demonstrate that RIPC, consisting of 4 cycles of 5 minutes ischaemia/5 minutes reperfusion of the upper limb, versus placebo control, reduced the incidence of CIN in high risk patients from 40% to 12% ( $p=0.002$ ). However, the surprisingly high rate of CIN in the control group, three times that seen in similar cohorts of patients<sup>5, 59, 422, 423</sup>, limits translation to widespread clinical use. The patient cohort studied had a high degree of LV systolic dysfunction and frequently required peri-procedural diuretic therapy, which may partially explain the high rate of CIN. In addition the investigators used LOCM (iohexol/omnipaque) which may be associated with greater likelihood of renal injury compared to IOCM (iodixinol/Visipaque)<sup>424, 425</sup>, as well as i.v. NaCl 0.9% hydration and oral NAC, which may be inferior to i.v. sodium bicarbonate<sup>426</sup>. Yamanaka et al<sup>427</sup> examined the effect of upper limb RIPC on CIN in patients undergoing PPCI for STEMI, a procedure conferring significantly increased CIN risk. RIPC was found to reduce the risk of CIN (as defined by a rise in SCr of  $>0.5\text{mg/dL}$  or  $>25\%$  at 48-72 hours), by 10% vs. 36% in the control group (OR 0.1, 95% CI 0.05-0.64,  $p=0.008$ ) as well as reducing short term MACE rate, although the study was not adequately powered for MACE outcomes.

Despite numerous small studies providing somewhat conflicting results (Table 15), two recent meta-analyses have found overall benefit for RIPC on CIN. Bei et al<sup>428</sup> examined 10 RCTs including 1389 patients undergoing CA or PCI and found that upper arm RIPC reduced CIN in all patients regardless of eGFR (OR = 0.52, 95% CI = 0.34-0.77,  $P = .001$ ), as well as 1 year MACE (OR = 0.36, 95% CI = 0.20-0.66,  $P < .001$ ), although interestingly lower limb RIPC was not effective. Zuo et al<sup>429</sup>, similarly examined 9 RCT's and found RIPC conferred a reno-protective effect (RR= 0.42; 95% CI, 0.27-0.65;  $P = 0.000$ ) without significant heterogeneity or other bias.

**Table 15: Summary of clinical trials investigating the effect of RIPC on CIN**

Study	Setting	RIPC CIN events	Control CIN events	Outcome RR, 95% CI
Er et al <sup>189</sup> 2012	Elective CA/PCI, eGFR<60ml/min	6/50	20/50	0.300 (0.132 – 0.684)
Igarashi et al <sup>62</sup> 2013	Elective CA/PCI, eGFR<60ml/min	2/30	8/30	0.250 (0.058 – 1.081)
Zagidullin et al <sup>430</sup> 2016	Elective CA/PCI	1/25	7/26	0.14 (0.019 to 1.112)
Yamanaka et al <sup>427</sup> 2015	PPCI in STEMI	5/47	17/47	0.294 (0.118 – 0.732)
Savaj et al <sup>406</sup> 2014	Elective CA/PCI in DM patients	1/48	5/48	0.200 (0.024 – 1.649)
Hoole et al <sup>431</sup> 2009	Elective PCI	6/104	10/98	0.54 (0.19 – 1.54)
<b>Negative or conflicting evidence</b>				
Menting et al <sup>432</sup> 2015	Elective CA/PCI eGFR<60ml/min	2/36	2/36	1.000 (0.149 - 6.718)
Balbir Singh et al <sup>407</sup> 2016	Elective PCI in DM eGFR<60ml/min	7/51	7/51	1.000 (0.373 – 2.679)
Xu et al <sup>433</sup> 2013	Elective PCI in elderly DM patients	4/102	3/98	1.28 (0.294 – 5.577)
Luo et al <sup>434</sup> 2013	Elective CA/PCI	2/101	1/104	2.059 (0.190 – 22.36)

## CHAPTER 2: AUDIT AND QUALITY IMPROVEMENT

### INTERVENTION

#### 2.1 CLINICAL AUDIT

In order to assess the feasibility of the study, a clinical audit was performed to assess the number of patients likely to meet the inclusion criteria, adherence to the Heart hospital CIN guidelines and the subsequent rate of observed CIN and all-cause mortality. This audit was carried out at The Heart Hospital from June 2011- May 2012 (12 months) which identified 1,913 patients as having undergone a coronary angiographic procedure.

The electronic patient record, CDR, was used to determine pre-procedure SCr and eGFR and at 48-72 hours afterwards. Further blood results were retrieved from the electronic TOMCAT database where data was not available on CDR (i.e. inter-hospital “treat and returns”). 312 of 1913 patients had a pre-procedural eGFR<60ml/min/1.73m<sup>2</sup> whose clinical records were then reviewed to determine whether appropriate CIN consent had been obtained, i.v. sodium bicarbonate 1.26% had been administered and repeat (48-72h) blood test requested. For all patients, 1 year mortality data was collected from CDR. The CIN rate was between 10% in outpatients with follow up blood tests and 22% in all patients, with or without follow up 48-72 hour blood tests, which is in keeping with the historical published rate of approximately 17%. Extrapolating from this data, we determined that approximately 300 patients per year would meet the eligibility criteria and assuming a 50% successful recruitment rate from this group, 150 patients per year could be recruited from the Heart Hospital alone. The results of this Audit were presented to stakeholders at UCLH and recommendations about changes to practice were made including adoption of a WHO style checklist. These presentations also served as platform to inform staff about the ERICCIN study prior to its commencement.

## 2.2 SAMPLE SIZE CALCULATION

At the time of the study design, the only previous study to evaluate RIPC and its effect on CIN suggested that it would be reasonable to expect a 70% reduction in the rate of CIN between preconditioned and non-preconditioned patients at 48 hours following contrast exposure<sup>189</sup>. Comparable studies in which RIPC reduces acute kidney injury following CABG surgery suggest that RIPC can reduce rates of AKI by up to 57%<sup>435</sup>. The rate of CIN in at-risk patients varies between cardiac centres and can be as low as 7% and as high as 40% in some studies<sup>189, 436</sup>, depending on patient characteristics and peri-procedural factors. From the Audit data the local CIN rate was estimated to be between 10% and 20%.

Using a **CIN incidence of 15%** and a **RIPC effect of a 60% reduction in CIN**, the following power calculation determined optimal sample size:

$$N_1 = \left\{ z_{1-\alpha/2} * \sqrt{\bar{p} * \bar{q} * \left(1 + \frac{1}{k}\right)} + z_{1-\beta} * \sqrt{p_1 * q_1 + \left(\frac{p_2 * q_2}{k}\right)} \right\}^2 / \Delta^2$$

$$q_1 = 1 - p_1$$

$$q_2 = 1 - p_2$$

$$\bar{p} = \frac{p_1 + k p_2}{1 + K}$$

$$\bar{q} = 1 - \bar{p}$$

$$N_1 = \left\{ 1.96 * \sqrt{0.105 * 0.895 * \left(1 + \frac{1}{1}\right)} + 0.84 * \sqrt{0.15 * 0.85 + \left(\frac{0.06 * 0.94}{1}\right)} \right\}^2 / 0.09^2$$

$$N_1 = 181$$

$$N_2 = K * N_1 = 181$$

$p_1, p_2$  = proportion (incidence) of groups #1 and #2,  $p_1 = 0.15, p_2 = 0.06$

$\Delta = |p_2 - p_1|$  = absolute difference between two proportions

$n_1$  = sample size for group #1,  $n_2$  = sample size for group #2

$\alpha$  = probability of type I error (usually 0.05)

$\beta$  = probability of type II error (usually 0.2)

$z$  = critical Z value for a given  $\alpha$  or  $\beta$

$K$  = ratio of sample size for group #1 to group #2

For a power of **80%** and  **$\alpha$  value of 0.05**, the study would need to recruit **362 patients**.

Potentially much larger numbers were required if the actual CIN rate was lower or the RIPC effect size smaller and as such the study was undertaken as a proof of concept, accepting that it may be underpowered. As part of the ethical approval for the study the REC requested that a Data Monitoring Committee review an interim analysis of the data, identified as after 100 patients had been recruited to the study, to ensure that the study was adequately powered and that no significant adverse events related to RIPC therapy in high risk patients had occurred.



# **CHAPTER 3: EFFECT OF REMOTE ISCHAEMIC PRECONDITIONING ON CONTRAST INDUCED NEPHROPATHY**

Contrast induced nephropathy (CIN) remains a common iatrogenic complication and is associated with adverse cardio-renal outcomes. Despite advances in protective therapies, the incidence of CIN in contemporary studies remains unacceptably high. Patients identified as being at high risk of CIN are particularly likely to benefit from additional reno-protective strategies, which must be administered rapidly and safely during urgent clinical scenarios such as acute coronary syndromes (ACS). This clinical trial was designed to evaluate whether a novel therapy known as remote ischaemic preconditioning (RIPC) is effective at reducing the incidence of CIN.

## **3.1 HYPOTHESIS**

Remote ischaemic preconditioning, in addition to optimal preventative therapies and procedural approaches, reduces the incidence of contrast induced nephropathy in at-risk patients undergoing contrast based cardiac procedures.

## **3.2 AIMS AND OBJECTIVES**

### **3.2.1 Overall aim**

To study the effect of RIPC on CIN and short term cardio-renal outcomes in a cohort of patients undergoing elective and urgent coronary angiography and PCI, who had been identified as being at increased risk of CIN due to persistent reduction in estimated glomerular filtration rate (eGFR) below 60ml/min/1.73m<sup>2</sup>.

### 3.2.2 Objectives

- 1) To study the effect of RIPC on CIN, defined as a 25% increase in serum creatinine (SCr) or eGFR, or an absolute rise in SCr of 0.5g/dl (44 $\mu$ mol/l) from baseline, at 48 hours post contrast medium exposure, in consecutive at-risk patients undergoing elective or urgent coronary angiography and PCI.
- 2) To study the effect of RIPC on persistent renal injury at 3 months following contrast exposure defined by chronic elevation in SCr or UACR.
- 3) To study the effect of RIPC on cardio-renal outcomes at 3 months following contrast exposure, defined as all-cause mortality, non-fatal MI, revascularisation, acute heart failure, non-fatal stroke, major haemorrhage, rehospitalisation, haemofiltration or haemodialysis during the follow up period.
- 4) To study the effect of RIPC on a sensitive marker of subclinical renal injury known as urine albumin creatinine ratio at 48 hours in consecutive at-risk patients undergoing elective or urgent coronary angiography and PCI.
- 5) To study the effect of RIPC on a novel biomarker of renal injury, known as NGAL at an early 6 hour time-point following contrast exposure in consecutive at-risk patients undergoing elective or urgent coronary angiography and PCI.
- 6) To study the effect of RIPC on CIN, novel biomarkers of renal injury, persistent renal dysfunction and short term cardio-renal outcomes in subgroups of patients with specific risk factors such as diabetes mellitus.

## CHAPTER 4: METHODS

### 4.1 ETHICAL APPROVAL AND INFORMED CONSENT

The study protocol and associated documents were drawn up in accordance with the International Conference on Harmonisation, Good Clinical Practice guidance which were subject to approval by the UCL Partners Joint Research Office (UCLP-JRO) committee for the ethics of human research. Approval was also sought, via the online IRAS application system, from NHS R&D as well as the Research Ethics Council (REC) and all study documents including consent forms, patient information leaflets and correspondence were submitted for review. Once approved, a local review was performed by the Heart Hospital research committee and a site specific information form was signed. Subsequent major amendments were individually submitted to UCLP JRO and REC for approval and local SSI forms at Basildon and East Surrey hospitals were also signed upon the commencement of multi-centre involvement.

#### **Trial Management:**

The Chief Investigator, Professor Derek Yellon was responsible for the day to day monitoring and management of the study at all sites and the UCLP JRO, on behalf of UCL as the Sponsor, was responsible for monitoring and auditing studies in its clinical research portfolio. The trial management committee consisted of Professor Derek Yellon, Dr Rob Bell and Dr R. Rear and the data monitoring committee (DMC) (see below). Auditing was conducted in accordance with the Department of Health Research Governance Framework for Health & Social Care (April, 2005), in accordance with the Sponsor's monitoring and audit policies and procedures. The study was enrolled onto the NIHR portfolio which supported clinical research nurse (CRN) activities.

### **Data Monitoring Committee:**

A condition for ethical approval by the REC was that a data monitoring committee (DMC) was to be formed in order to analyse the interim dataset of the first 100 patients recruited, to ensure that the study was appropriately powered and to investigate RIPC safety in high risk groups. This was chaired by Dr Malcolm Walker, Clinical Director of the Hatter Cardiovascular Institute, Professor Raymond Macallister, an independent expert at UCL and Dr Tim Clayton, an independent statistical expert at the London School of Hygiene and Tropical Medicine.

### **Safety issues:**

RIPC is known to be a low risk intervention with no major adverse events demonstrated in a number of clinical trials, although there is a small risk of minor bruising or transient skin discoloration to the area of the limb on which the cuff is placed. There is a very small risk during venepuncture for the transmission of blood borne diseases.

### **Patient Withdrawal**

We ensured that participants knew that they were free to withdraw their consent at any time during the trial, without reason and without prejudicing their usual care. Although not an occurrence during the study, patients losing the ability to consent during the trial would have been withdrawn, however samples and data collected whilst they remained able to consent may have been used.

## **UCL Indemnity Statement:**

*“UCL holds insurance against claims from participants for harm caused by their participation in this clinical study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, if this clinical study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical study. University College London does not accept liability for any breach in the hospital’s duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.”*

## **Reporting Serious Adverse Events**

All Serious Adverse Events (SAE’s) in the study were reported immediately to the sponsor by email as well as via the UCLH ‘Datix’ system, and were documented from the point of enrolment until the patient had exited from study including a comprehensive description of the event.

## **Registration of Clinical trial**

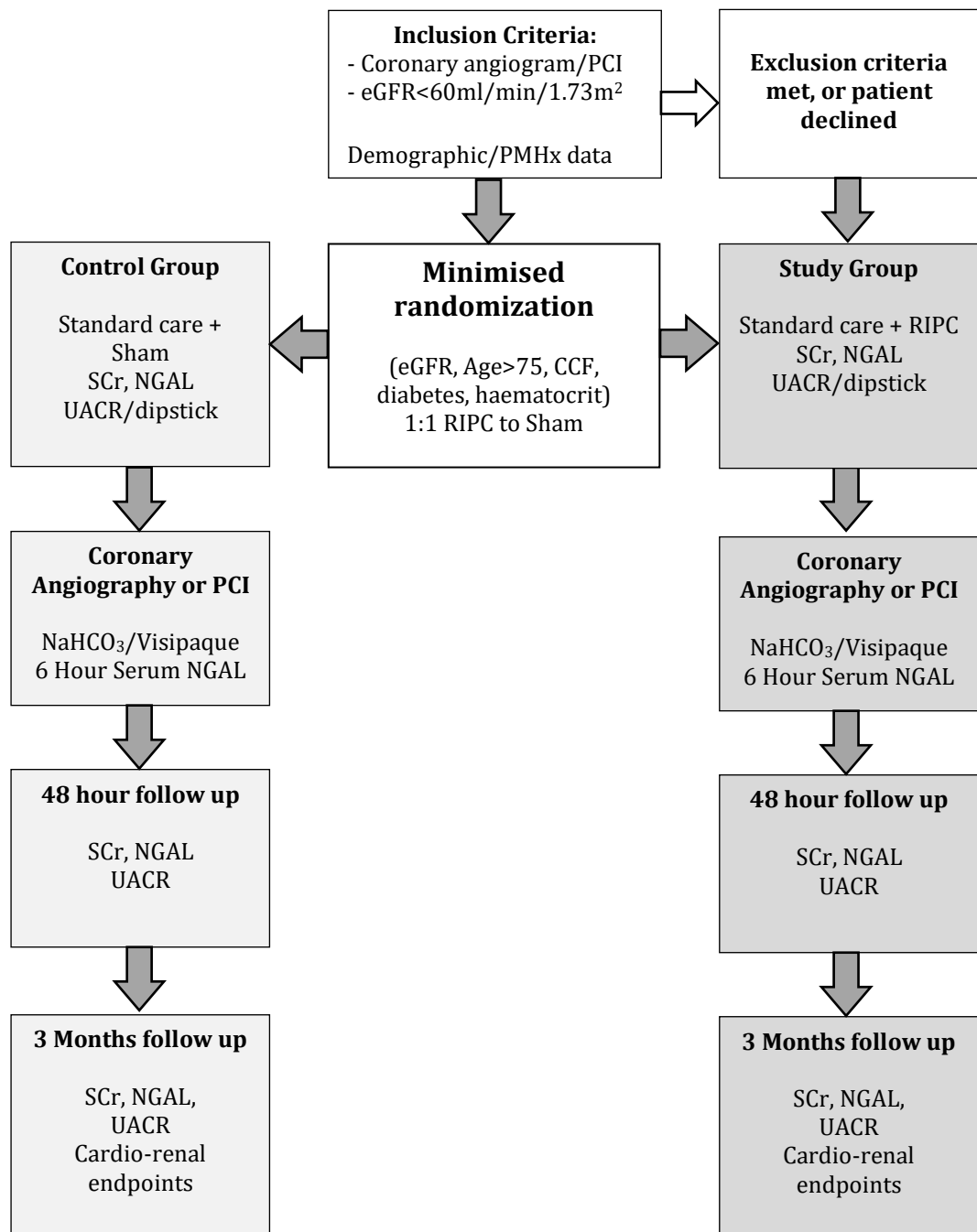
Following successful REC (13/LO/0502) and local NHS R&D (12/0578) approvals ERICCIN was registered with ISRCTN, registry no. 49645414.

## 4.2 STUDY DESIGN AND PATIENT RECRUITMENT

The study was initially conceived as a single centre double blinded randomized placebo controlled trial at the Heart Hospital, UCLH, and was subsequently expanded to a multi-centre study including two further research centres, Basildon Hospital and East Surrey Hospital. Consecutive patients awaiting elective coronary angiography or PCI for stable anginal symptoms or urgent PCI for NSTEMI were screened by a member of the patient's usual cardiology team based upon the study inclusion and exclusion criteria, using elective and emergency cardiac procedure lists and the electronic patient records for demographic and biochemical data (Figure 9).

Patients received verbal and written information about the study from their usual cardiology team in the form of a face to face or telephone introduction and patient information sheet (Appendix 1). Patients waiting for an elective procedure were given approximately 1 week and those waiting for urgent procedures at least 24 hours to consider participating in the study. For those interested in participating, an interview with a researcher was conducted on the day of the procedure in order to collect demographic and clinical data and to answer any questions. If the patient was willing and able to provide informed consent this was documented using the study consent form by a researcher with a current certificate in Good Clinical Practice. For non-English speakers, this interview took place with an independent interpreter present or via a telephone service. A copy of the consent form was given to the patient, another filed in the clinical notes and the original was stored securely in the participants study folder. No monetary payment of participants or researchers occurred other than the occasional reimbursement of travel costs. Following enrolment a standard letter was sent to patients GP's confirming participation in the study.

**Figure 9: ERICCIN study design diagram**



PCI – Percutaneous coronary intervention, eGFR – estimated glomerular filtration rate, CCF – congestive cardiac failure, SCr – serum creatinine, NGAL – neutrophil gelatinase associated lipocalin, ACR – albumin creatinine ratio

### 4.3 INCLUSION AND EXCLUSION CRITERIA

#### **Inclusion Criteria:**

- Age 18 to 85.
- Male or Female gender.
- Awaiting elective or urgent coronary angiography or PCI
- eGFR <60ml/min/1.73m<sup>2</sup> (MDRD)

#### **Exclusion criteria:**

- Age less than 18 or over 85
- Inability to give written informed consent.
- Pregnancy
- Patients receiving haemo or peritoneal dialysis
- Significant upper limb peripheral arterial disease
- Other contraindication to BP cuff inflation (friable skin/axillary lymph node clearance etc.)
- Coagulopathy with INR >2.0
- Participation in another interventional clinical trial within 3 months.
- Intravascular contrast exposure within one month prior to study date.
- ST elevation MI/Cardiac arrest/Cardiogenic shock during admission.



#### 4.4 RANDOMISATION METHOD & BLINDING

Minimised randomisation was utilised with minimisation factors of stratified eGFR (<20ml/min, 20-40ml/min, 40-60ml/min), age (<75yr, >75yr), diabetes, NYHA III/IV heart failure and haematocrit (<0.36 females, <0.39 males) as the pre-procedural weighted predictors of CIN identified by Mehran et al<sup>52</sup>. This ensured that the study and placebo groups were evenly matched in terms of the pre-procedure risk of CIN. Patients were assigned in a 1:1 ratio to either RIPC or placebo. This was performed by a researcher using a freeware computer programme 'MinimPy 0.3' on a secure laptop (0). A study number and a treatment group letter (A or B) was generated and these results were documented on a confidential list to allow future data analysis or unblinding during the study if necessary.

To ensure blinding, the researcher was not aware which group letter corresponded with the RIPC treatment or placebo arms and the automated RIPC device (DERIC) administered a predetermined therapy based on this letter, again unknown to the researcher. The placebo arm involved 5 minute cycles of sub-therapeutic inflation of the blood pressure cuff to 10mmHg followed by deflation to 0mmHg. As such the researcher, clinical team and patient were blinded as to whether RIPC therapy or placebo had been delivered. All data analysis was carried out by a blinded fellow and no serious adverse events occurred necessitating unblinding of the researchers or patients.

Figure 10: 'Minimpy' minimised randomisation software

The screenshot displays the 'Minimization Program [ERICCIN]' software interface. The main window contains a table with the following columns: #, UI, Group, AGE>75, CCF, DIABETES, HCT<0.39, and eGFR. The table lists 24 rows of data. An 'Allocation Add' dialog box is open in the center, titled 'Allocation Add'. The dialog box contains the text 'Select variables level from menus below' and a list of variables with their corresponding levels selected in dropdown menus: AGE>75 (<75), CCF (NO CCF), DIABETES (DM), HCT<0.39 (NO ANAEMIA), and eGFR (<20). Below the dropdown menus, there is a section for 'New case extra properties' with 'Name' and 'Value' fields. The dialog box also includes 'Add', 'Delete', 'Date', and 'Cancel' buttons.

#	UI	Group	AGE>75	CCF	DIABETES	HCT<0.39	eGFR
0	140	A	<75	NO CCF	NO DM	NO ANAEMIA	40-60
1	261	A	<75	CCF	NO DM	ANAEMIA	20-40
2	282	B	<75	NO CCF	DM	NO ANAEMIA	40-60
3	306	B	<75	NO CCF	DM	NO ANAEMIA	40-60
4	246	A	<75	NO CCF	DM	ANAEMIA	40-60
5	187	B	<75	NO CCF	DM	NO ANAEMIA	20-40
6	062	A	<75	NO CCF	DM	ANAEMIA	40-60
7	097	B	<75	NO CCF	DM	NO ANAEMIA	40-60
8	053	B	<75	NO CCF	DM	ANAEMIA	40-60
9	158	A	<75	NO CCF	DM	ANAEMIA	20-40
10	336	B	<75	NO CCF	DM	ANAEMIA	20-40
11	095	A	<75	NO CCF	DM	ANAEMIA	20-40
12	115	A	<75	NO CCF	DM	NO ANAEMIA	20-40
13	276	B	<75	NO CCF	DM	ANAEMIA	20-40
14	352	B	<75	NO CCF	DM	ANAEMIA	20-40
15	284	B	<75	NO CCF	DM	ANAEMIA	20-40
16	007	A	<75	NO CCF	DM	NO ANAEMIA	40-60
17	186	B	<75	NO CCF	DM	ANAEMIA	20-40
18	159	A	>75	NO CCF	DM	NO ANAEMIA	20-40
19	211	A	>75	NO CCF	DM	ANAEMIA	20-40
20	202	B	<75	CCF	DM	NO ANAEMIA	40-60
21	265	B	<75	NO CCF	DM	ANAEMIA	40-60
22	318	B	<75	NO CCF	NO DM	ANAEMIA	20-40
23	270	A	>75	NO CCF	DM	ANAEMIA	40-60

## 4.5 CIN PREVENTION PROTOCOL

The established UCLH CIN prevention protocol (Figure 11) was used for all enrolled patients, which included encouragement of oral hydration, diuretic and nephrotoxic withdrawal for 24 hours (NSAID, aminoglycoside, cyclosporine, and amphotericin B), metformin withdrawal for 48 hours, intravenous pre-hydration with Sodium Bicarbonate 1.26% at 3ml/kg/hour for 1 hour pre-procedure and 1ml/kg/hour for 6 hours post procedure, use of iso-osmolar ‘Visipaque’ contrast medium, minimisation of contrast volume and use of biplane imaging where appropriate.

**Figure 11: CIN prevention protocol at UCLH**

<u>Sodium Bicarbonate prevention of Contrast Nephropathy for patients undergoing coronary intervention, AF ablation and Biventricular devices</u>		
<ul style="list-style-type: none"> <li>All elective patients undergoing contrast studies should have their serum creatinine and eGFR calculated.</li> <li>All emergency patients, where serum creatinine is unknown, should receive Visipaque.</li> <li>Metformin: patients should discontinue 48 prior to procedure. This can be re-started 48hrs post procedure as long as there is no evidence of renal function decreasing.</li> <li>All patients should avoid diuretics, NSAID's and aminoglycosides on the day of the procedure.</li> <li>All patients with a history of cardiac failure will receive visipaque.</li> </ul>		
	eGFR > 60ml/min	eGFR <60ml/min
Avoid dehydration, encourage oral fluids	✓	✓
Include risk of CN in consent	✓	✓
Minimise contrast load	✓	✓
Consider alternative imaging		✓
IV hydration Using NaHCO <sub>3</sub> as per protocol opposite:		✓
Use iodixanol (Visipaque)		✓
Check blood results prior to discharge		✓
Arrange follow up bloods 48-72 hours post procedure		✓

**Sodium Bicarbonate protocol**

- NaHCO<sub>3</sub> @ 3ml/kg/hr for 1 hr pre-procedure
- NaHCO<sub>3</sub> @ 1ml/kg/hr for 6 hrs post-procedure

**Solution: 1.26% Sodium Bicarbonate (Polyfusor 500ml)**

*If the first hour is completed, and the lab are not ready to receive the patient, then commence the 6 hr post-procedure infusion and patients can have their procedure with the infusion in situ.*

## **4.6 TRANSIENT LIMB ISCHAEMIA PROTOCOL**

The RIPC or sham procedure was performed by a blinded researcher aiming for a window two hours prior to contrast exposure, although an exact pre-treatment window was not possible due to the constraints of variable caseloads and emergencies in the catheter laboratory. The patients' right arm was inspected and a baseline blood pressure reading was made to ensure it did not exceed 200mmHg. The RIPC protocol involved inflation of a standard size BP cuff on the right upper arm to 200mmHg for five minutes, followed by 5 minutes cuff deflation to 0 mmHg. A total of four cycles of inflation and deflation was performed. The sham procedure involved cuff inflation to 10mmHg for five minutes, followed by deflation to 0mmHg for five minutes with four cycles. The RIPC and sham therapies were visually and audibly indistinguishable to the researcher and clinical team. This upper limb RIPC protocol has been widely used in many studies and is known to be a safe and tolerable intervention, as demonstrated in a recent RCT performed by Sharma et al<sup>457</sup> where patient reported pain scores were between 4 and 5 on a 'likert' pain scale ranging between 0 (least) and 10 (greatest).

## **4.7 CORONARY ANGIOGRAPHY AND PCI PROCEDURE**

Coronary angiography or PCI was performed as per usual by blinded clinicians. Relevant data was collected including the volume and type of contrast used, whether the procedure included diagnostic coronary angiography alone, LV/Aortogram, PCI procedure and any intra-procedural complications such as hypotension (Systolic BP<90mmHg) or intra-arterial balloon pump (IABP) therapy. The use of biplane imaging was left to the discretion of the blinded coronary angiography operator.

## 4.8 CIN BIOMARKERS & CLINICAL OUTCOMES

Prior to i.v. sodium bicarbonate 1.26% administration and contrast exposure, a blinded researcher or phlebotomist at the Heart Hospital collected venous blood for baseline SCr and serum NGAL tests during insertion of an intravenous cannula, and urine sample was collected for dipstick analysis and UACR. Following the procedure a blinded researcher or phlebotomist at The Heart Hospital/UCLH subsequently collected a 6 hour serum NGAL sample. A small cohort of patients also had 'point of care' NGAL samples measured at 0 and 6 hours. The 48 hour and 3 month SCr, serum NGAL and UACR samples were collected at the Heart Hospital or UCLH. For patients with poor mobility these samples were collected at nominated satellite research sites including the North Middlesex, Whittington and Homerton hospitals and couriered immediately for analysis at the UCLH laboratory.

SCr analysis was performed using the Roche Cobas modular analyser series (Roche Diagnostics, Burgess Hill, UK). A rate blanked, compensated Jaffe method was used for all samples. UACR was analysed using quantitative particle enhanced immune-inhibition method (Dade Behring aca IV® analyser, Dade Behring Inc., Wilmington, DE). NGAL point of care analysis was performed using Alere Triage® fluorescence immunoassay NGAL Test strips and Alere Triage® Meter.

At 3 months a blinded researcher performed telephone interviews with patients and the patients' GP to document changes to medications and identify cardio-renal endpoints that may have occurred.

## **4.9 PRIMARY OUTCOME MEASURE**

The incidence of CIN, as defined by a relative 25% increase, or 0.5g/dl (44 $\mu$ mol/l) absolute increase in serum Creatinine from baseline at 48 hours post contrast medium exposure.

## **4.10 SECONDARY OUTCOME MEASURES**

The change in serum creatinine, eGFR and urine albumin creatinine ratio (UACR) from baseline to 48-hours and 3 months post contrast medium exposure.

The change in serum neutrophil gelatinase-associated lipocalin (NGAL) from baseline to 6 hours, 48 hours and 3 months post contrast exposure.

The assessment of short term cardio-renal endpoints including death, non-fatal MI, revascularisation, acute heart failure, non-fatal stroke, major haemorrhage, rehospitalisation, haemofiltration or haemodialysis during three months follow-up.


## 4.11 DATA COLLECTION, HANDLING & RECORD KEEPING

A blinded fellow or research nurse used a case report form (CRF) to perform data collection. This confidential document identified the patient with a unique study number, initial, date of birth, documented fulfilment of the inclusion and exclusion criteria, randomisation data, baseline demographic and medical history and medications. Numerical data was entered onto the CRF for serum creatinine, serum NGAL, and urine dipstick/UACR at the various time points. The CRF was then transcribed by a blinded researcher or research nurse onto a secure UCL database, known as 'Redcap' to allow for data collection across the multicentre research sites and to facilitate analysis (Figure 12).

For each participant, the CRF was stored in an individual patient folder with separate patient identifiable information such as name, address, GP details and contact telephone number. The folders were secured in a locked room at The Heart Hospital and subsequently at The Hatter Cardiovascular Institute, UCL. Patient identifying information was not used in data analysis after participants were allocated a unique study number. All computerized data was stored on password-protected computers in encrypted drive partitions. Some routine patient data, such as blood test results, was available to the clinical team via password-protected electronic patient record.

In accordance with its current Records Retention Schedule, research data is retained by UCL as sponsor for 20 years after the research has ended. The UCL Records Office provides a service to UCL staff and maintains records in a safe and secure offsite location and access to stored records is strictly controlled.

Figure 12: Online 'Redcap' case report form database



Logged in as rrear | Log out

My Projects  
Project Home  
Project Setup  
Project status: **Production**

**Data Collection**

Record Status Dashboard  
Add / Edit Records

**Applications**

- Calendar
- Data Export Tool
- Data Import Tool
- Data Comparison Tool
- Logging
- Field Comment Log
- File Repository
- User Rights and DAGs
- E-signature and Locking Mgmt
- Graphical Data View & Stats
- Data Quality
- Report Builder

**Help & Information**

- Help & FAQ
- Video Tutorials
- Suggest a New Feature

If you are experiencing problems, please

### ERICCIN

**Record Status Dashboard (all records)**

Displayed below is a table listing all existing records/responses and their status for every data collection instrument (and if longitudinal, for every event). You may click any of the colored buttons in the table to open a new tab/window in your browser to view that record on that particular data collection instrument. Please note that if your form-level user privileges are restricted for certain data collection instruments, you will only be able to view those instruments, and if you belong to a Data Access Group, you will only be able to view records that belong to your group.

Displaying record "000" through "2326" of 85 records

REDCap Subject Code	ERICCIN CRF Admission	ERICCIN CRF 48 Hours 48 Hour bloods	ERICCIN CRF 3 Months 3 month bloods/telephone
000	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>
007	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>
009	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
012	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>
018	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>
020	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>
023	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>
035	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>
037	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>
053	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>
062	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>
067	<input checked="" type="radio"/>	<input checked="" type="radio"/>	<input checked="" type="radio"/>

**Legend for status icons:**  
 Incomplete     Incomplete (no data saved)  
 Unverified  
 Complete



## 4.12 STATISTICAL ANALYSIS

Variables were tested for normal distribution using the Shapiro Wilk test, data that conformed to a normal distribution were summarised as means (SD) and otherwise as median and first and third quartiles (Q1–Q3). The effect of RIPC treatment on CIN was evaluated using logistic regression, adjusted for the minimization factors, as well as other covariates of interest (gender, volume of contrast, peri-procedural hypotension and Intra-Arterial Balloon Pump (IABP) use). The effect of RIPC treatment on serum creatinine change, eGFR(MDRD) change, serum NGAL and UACR compared at the two time points was evaluated using the Mann-Whitney U test. Statistical analyses were performed with SPSS 22.0. A 2-sided probability value of 0.05 was considered to indicate statistical significance.

The data analysis was performed blinded and the results were summarised in tables and box and line graphs. The tables include demographical, clinical and procedural data, as well as the results of blood and urine tests taken at the three time points. A table displays data relating to the primary and secondary outcomes as well as subgroup analysis of patients with higher CIN risk. Line graphs have been used to display surrogate measures for CIN at different time points in study patients and control patients. Missing data due to withdrawal or non-compliance has been documented in the results.

## **4.13 MAJOR AMENDMENTS TO THE PROTOCOL**

### **22/11/13 – Version 1.2 Approved by REC**

The study inclusion criteria were amended to include patients with impaired renal function undergoing biventricular pacemaker insertion using intravascular contrast medium. This amendment was aimed to increase the number of potentially eligible patients for the study. Clarification of the recruitment and randomisation methods was made and the analysis of serum Cystatin C and urinary NGAL was removed from the protocol given that neither investigation added significantly to the scientific validity or safety of enrolled participant and incurred significant costs. Three satellite blood test collection centres were included in the study to facilitate 48-hour and 3-month sample collection for patients with impaired mobility, namely the North Middlesex, Homerton and Whittington hospitals.

### **29/5/14 Version 1.3 approved by REC**

In order to increase recruitment and to widen participant demographics, the study was expanded to a multicentre trial including Basildon Hospital (PI Dr Reto gamma) and the East Surrey Hospital (PI Dr Shrilla Banerjee). The London Chest Hospital, The Royal Free Hospital and Barnet Hospital were also approved for inclusion into the study however were unable to participate due to difficulty in securing staff for NIHR nursing support. Site-specific patient information sheets, consent forms and GP letters were approved. Grant funding of £37,266 from the NIHR UCL Biomedical Research Centre was confirmed.

### **15/5/15 Version 1.3.1 Amendment to laboratory NGAL analysis**

A small cohort of patients (n=10) had a 'point of care' (POC) serum NGAL testing at 0 and 6 hours post procedure in order to assess whether changes in serum NGAL from baseline might predict CIN at the bedside. It was initially planned that these results were to be correlated with formal laboratory NGAL samples collected on all patients to ensure validity. Following the DMC review of the study and the conclusion that the study was underpowered and had not demonstrated any significant difference in the primary outcome measure between groups, it was decided that formal laboratory NGAL analysis on all patients would not add significantly to findings of the study. It would however have increased study costs substantially and therefore the formal laboratory NGAL results were not processed. The results from the NGAL point of care tests have been presented in the results section below. No patients who had POC NGAL analysis went on to develop CIN.

#### 4.14 DEVELOPMENT OF THE ‘DERIC’ DEVICE

In order to facilitate the administration of the RIPC or sham procedure by a blinded researcher and to allow for precise delivery of the RIPC protocol, an automated RIPC device was designed and built in collaboration with the UCL biomedical department. The initial design of this device consisted of dual blood pressure cuffs that would uniquely enable the automated delivery of RIPC at two different limb sites simultaneously thus potentially halving the time needed for effective treatment. This device was named the ‘Dual electronic remote ischaemic conditioning’ device, or ‘DERIC’ (Figure 13).

This particular ‘dual conditioning’ ability was not specifically utilised in the ERICCIN trial although it was hoped that this would be of benefit in future studies. Much of the hardware of the DERIC device was derived from existing automated blood pressure measurement machines, as well as utilising ‘off the shelf’ electronics, which greatly reduced the cost and time involved in developing the prototype (Figure 14). The hardware is controlled by a laptop PC with a user-friendly graphical user interface (Figure 15) that allows the researcher to easily configure the therapy that is required and allows objective data collection on the treatment that has been delivered (Figure 16). Although the DERIC device was not submitted for CE marking, not being for commercial use, the device underwent a formal safety assessment, performed by Medical Engineering Solutions Ltd prior to its use in the study (see Appendix).

Two further single pump laptop controlled devices were manufactured based upon the original specification for use at Basildon Hospital and East Surrey Hospital research sites and it is hoped that these devices will also be utilised in future RIPC research studies at the Hatter Cardiovascular Institute, UCL.

Figure 13: Illustration and picture of the 'DERIC' device

## DERIC Device

### Dual Electronic Remote Ischaemic Conditioning

*Designed & built in partnership with the UCL Medical Physics and Bioengineering department*

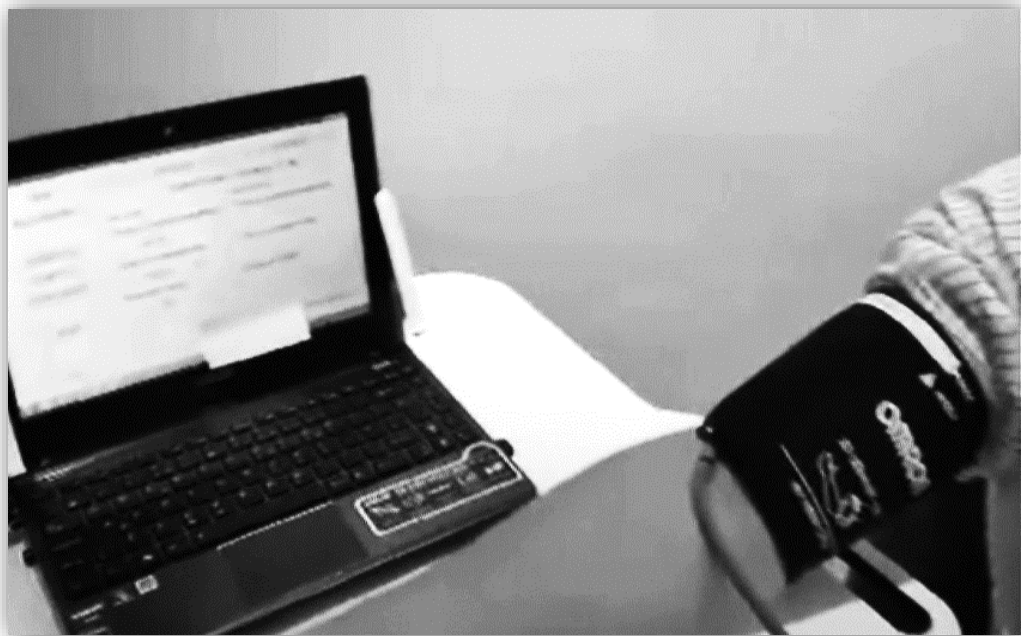
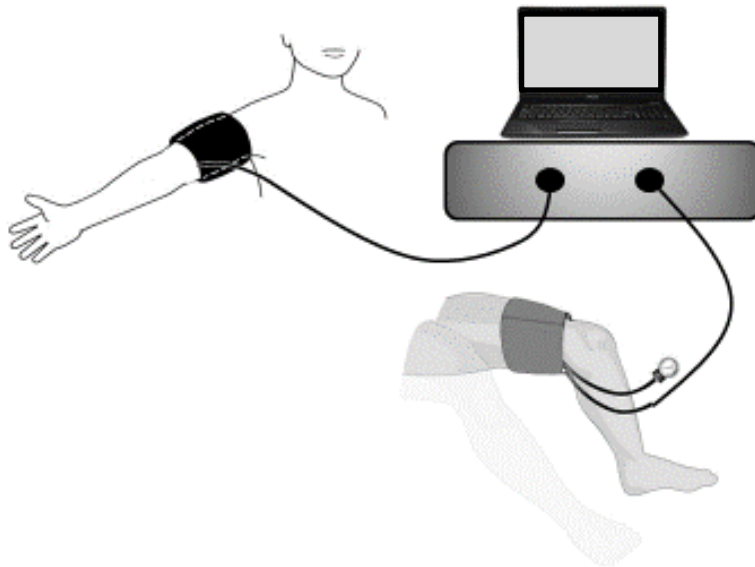


Figure 14: Block diagram of the 'DERIC' device

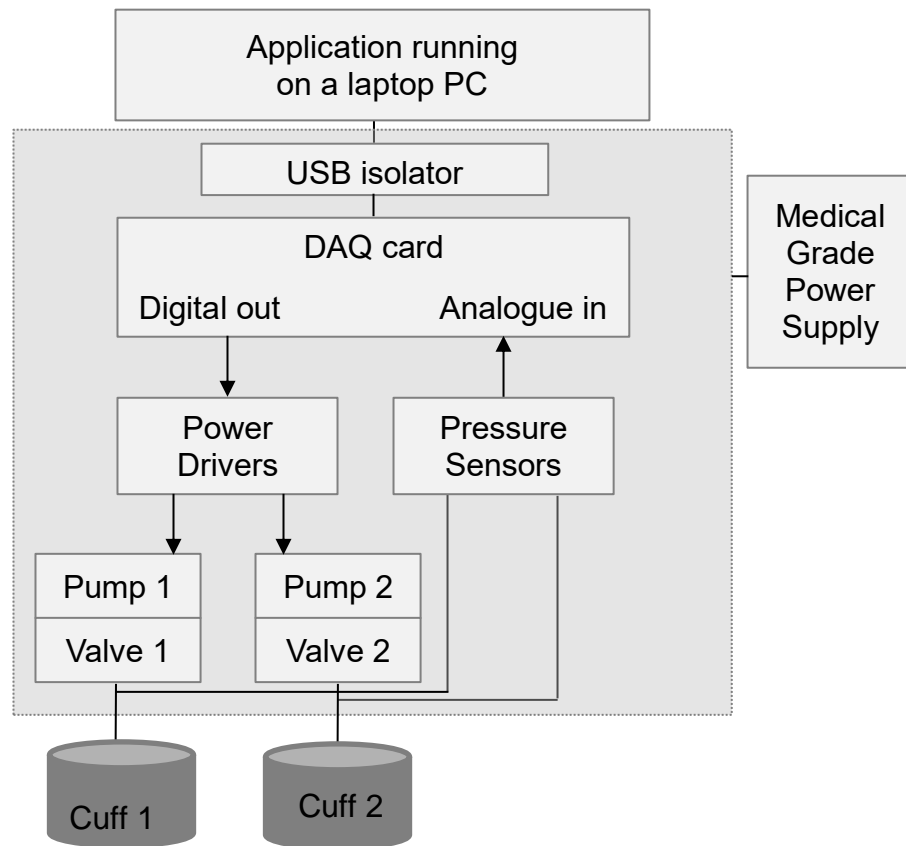


Figure 15: DERIC graphical user interface control

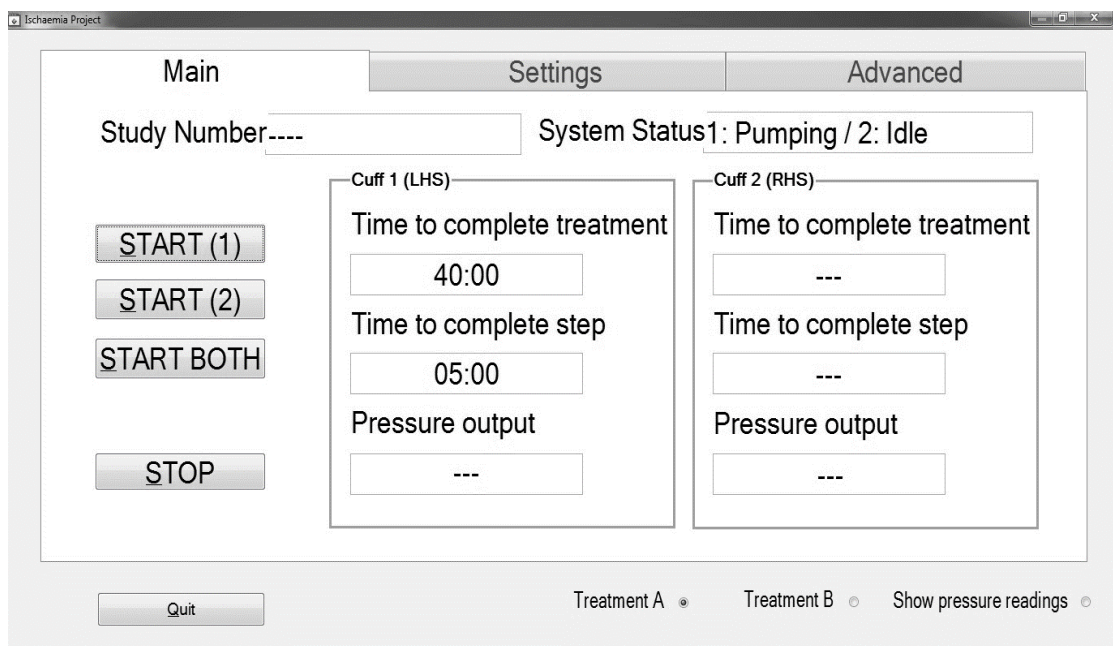


Figure 16: Database for the DERIC RPC/Sham delivery

**DATABASE SEARCH**  
Selected 2347 Logs.

Search start date

Search end date

Inflating-start	03/09/13	09:41:15	001
Occlusion	03/09/13	09:41:30	001
Inflating-midstart	03/09/13	09:42:32	001
Inflating-midstop	03/09/13	09:42:33	001
Inflating-midstart	03/09/13	09:45:06	001
Inflating-midstop	03/09/13	09:45:07	001
Rest	03/09/13	09:46:31	001
Inflating-start	03/09/13	09:51:32	001
Occlusion	03/09/13	09:51:43	001
Inflating-midstart	03/09/13	09:52:05	001
Inflating-midstop	03/09/13	09:52:06	001
Inflating-midstart	03/09/13	09:54:03	001
Inflating-midstop	03/09/13	09:54:04	001
Rest	03/09/13	09:56:44	001
Inflating-start	03/09/13	10:01:45	001
Occlusion	03/09/13	10:01:56	001
Inflating-midstart	03/09/13	10:02:29	001
Inflating-midstop	03/09/13	10:02:30	001
Inflating-midstart	03/09/13	10:04:40	001
Inflating-midstop	03/09/13	10:04:41	001

## 4.15 PARTICIPANT FOCUS GROUP

In order to optimise recruitment to the study a patient focus group meeting was held at the midpoint of the study in order to assess participant experience and to receive feedback. 42 participants recruited to date were approached and two separate small group meetings for both control (n=7) and RIPC (n=6) participants were held at the Hatter Cardiovascular institute on the 17<sup>th</sup> July 2014. By holding separate meetings, participants were prevented from comparing their treatment regimens with one another and in doing so blinding was maintained. The meeting was chaired by two independent researchers, Dr R. Bell and Ms K Bavnbek, and participant responses to structured questions and an open feedback session were recorded to audio tape and transcribed.

### **A) Initial contact with research team and Information sheet**

- How were you initially approached by the research team?
- Was this convenient for you and was the team member polite and helpful?
- Was the information given to you clear and concise?
- Was the need to return to hospital for blood tests after your procedure explained to you?
- Did we offer blood test collection at your local hospital?
- Were you given an opportunity to ask questions or raise any concerns?
- Did you feel under any undue pressure to participate in the study?
- Were you given the contact details of the research team?



## **B) On the day of the procedure**

- Was the study re-explained to you and were you given the opportunity to ask questions?
- Were the research team polite and helpful?
- How did you find the remote ischaemic conditioning procedure?
- Did you notice any ill effects afterwards?
- Were you given clear information about how to have your blood tests collected?
- Were you given the contact details of the research team in case of any problems?

## **C) Follow up after your procedure**

- How convenient for you was the 48 hour and 3 month blood collection?
- Did you know when and where to have your blood collection?
- Did you experience any problems whilst attending for your blood collection?
- If so were you able to communicate these to the research team?
- How could we improve this process for you?
- Was there anything you wished you had been told before joining the study?
- Were you told you would receive feedback on the results of the study?

## **D) Open feedback session**

#### **4.16 REPORTING AND DISSEMINATION OF RESULTS**

An anonymised data summary sheet will be offered to participants and the Sponsor at the end of the study. The anonymised data has also been summarised and presented at internal meetings at the Hatter Cardiovascular Institute as well as at the participating hospitals. It is hoped that in addition to the benefit of this thesis, the anonymised data will be used by the author for submission to peer-reviewed journals for publication, as well as being presented at local and national Cardiovascular and Renal conferences. The rationale for the study was published in a peer reviewed journal at the outset of the clinical trial.

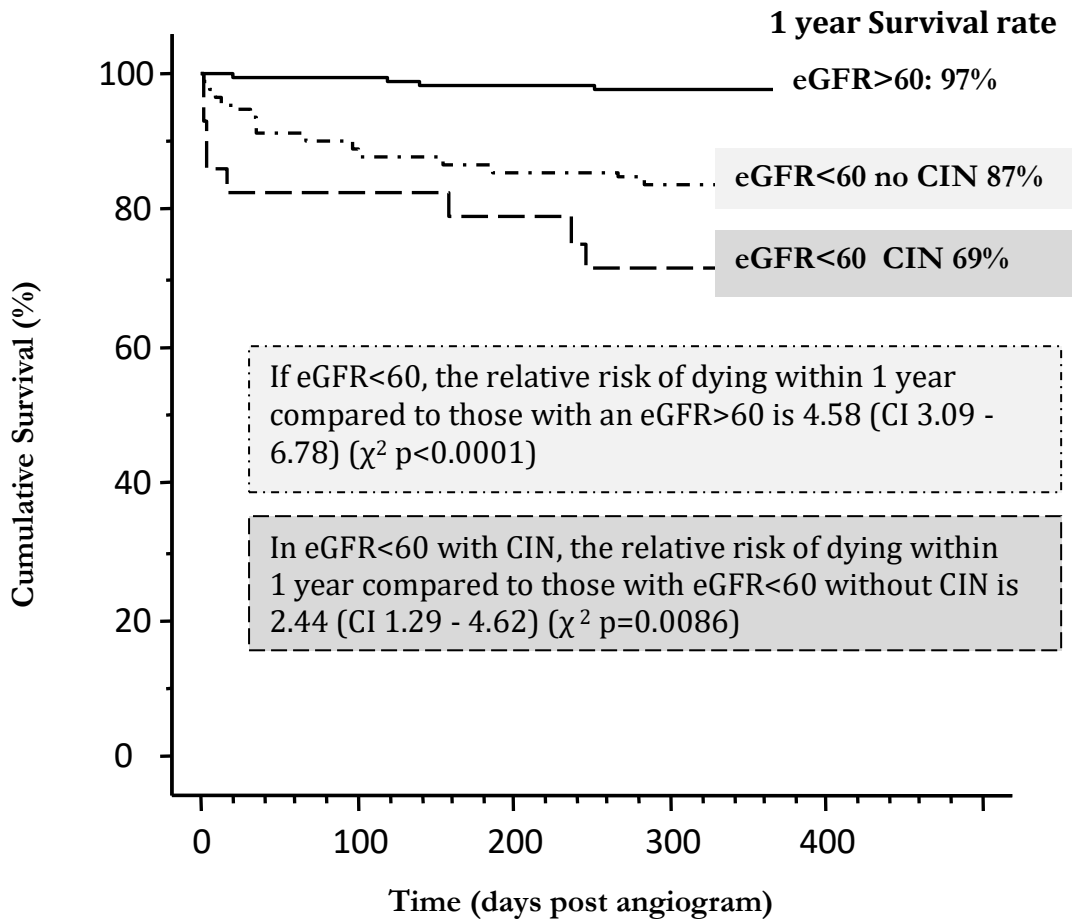
## CHAPTER 5: RESULTS

### 5.1 CLINICAL AUDIT

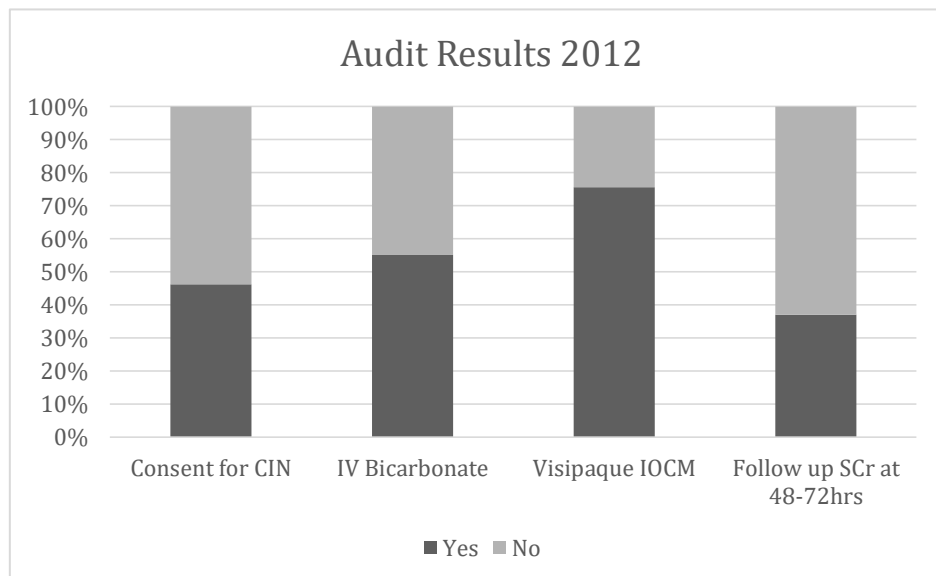
- 1,913 patient events encoded “*Coronary angiography*” identified.
- Mean patient age was 64, 71% were male.
- 25.3% were “*treat and returns*” with no recorded blood test data on the UCLH CDR (half of these patients had data recorded on the “TOMCAT” angiographic database and therefore could be included in the audit).
- 1,773 had no follow-up within the UCLH NHS trust.
- 312 patients were identified as having an eGFR<60 (giving a prevalence of 19%).
- Case notes of 221 patients with an eGFR<60ml/min (72%) were reviewed.
- Of the 312, only 141 had a 48h blood test within the Trust, however this identified 31 cases of contrast induced nephropathy.
- Overall rate of CIN in the eGFR<60 ml/min group was 10%.
- CIN rate for those with repeat blood within the trust was 22%.
- The 1 year survival rate for patients with an eGFR<60ml/min and CIN was 69%, compared with 87% for patients with an eGFR<60ml/min and no CIN and 97% for those with an eGFR>60ml/min (Figure 17).

**Figure 17: Kaplan-Meier Cumulative 1 Year Survival Plot**

All-cause mortality of patients undergoing coronary angiographic procedures at UCLH, June 2011 to July 2012 (n=1,913)

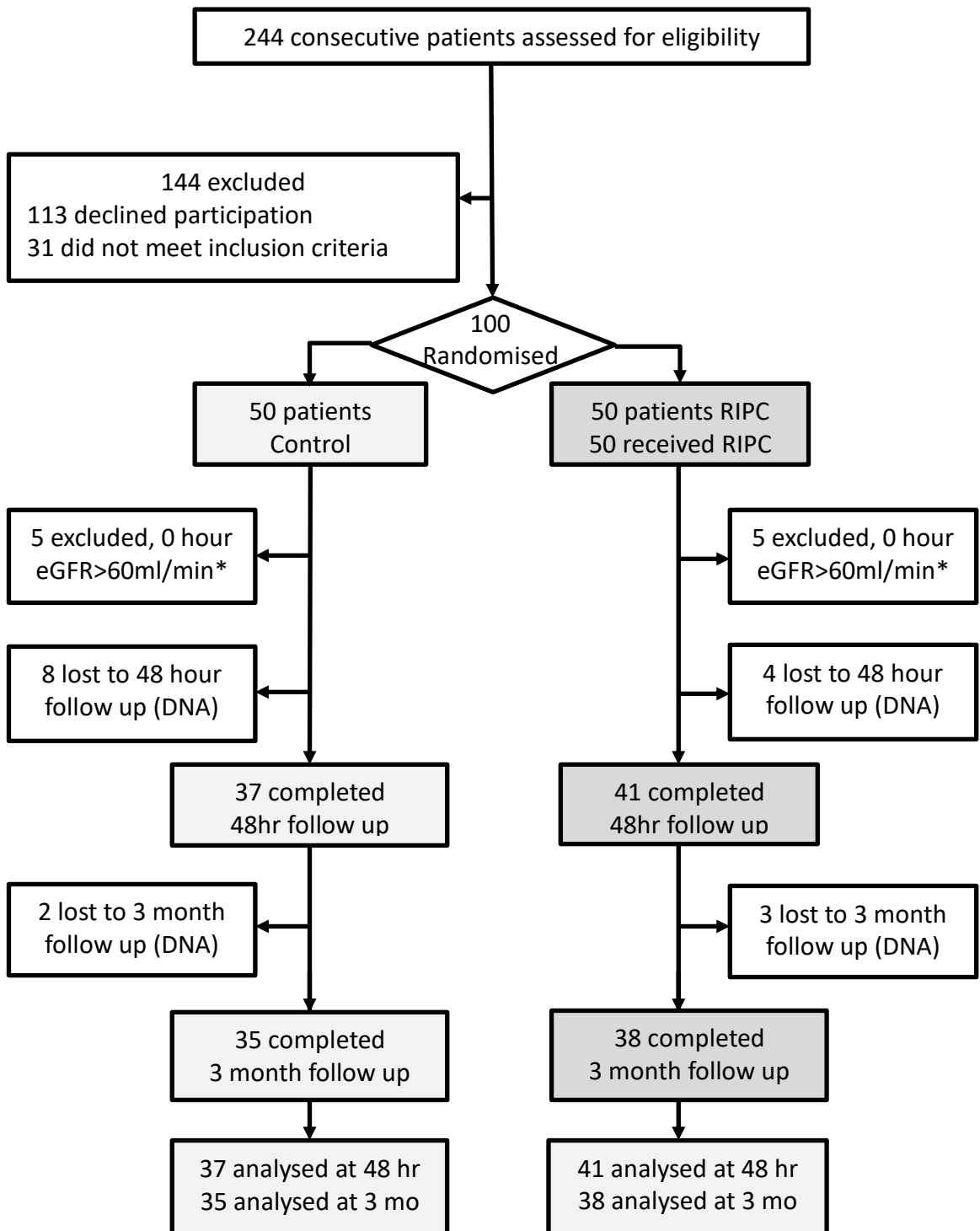


**Figure 18: Results of 2012 Audit case note review**



## 5.2 PATIENT RECRUITMENT

Figure 19: Study CONSORT diagram



\* Patients with 0 hour eGFR >60ml/min, despite a screening eGFR <60ml/min, were excluded due to the definition of CKD, requiring two sequential eGFR measurements of <60ml/min within 3 months

### 5.3 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

**Table 16: Demographic, clinical and angiographic characteristics**

	<b>Control Group (n=37)</b>	<b>RIPC Group (n=41)</b>	<b>P value**</b>
Age, years (SD)	71.68 (+/-8.54)	72.12 (+/-8.137)	0.81
Age > 75*	13 (35.1%)	16 (39%)	0.54
Gender (Male)	26 (70.3%)	26 (63.4%)	0.52
Body Mass Index, Kg/m2 (SD)	30.39 (+/-6.31)	29.16 (+/-3.41)	0.28
Hypertension	29 (78.4%)	35 (85.4%)	0.42
Smoking History	27 (72.9%)	27 (65.9%)	0.49
Dyslipidaemia	27 (72.9%)	33 (80.5%)	0.43
Diabetes Mellitus*	18 (48.6%)	19 (53.7%)	0.84
Peripheral Arterial Disease	3 (8.1%)	3 (7.3%)	0.89
Acute Coronary Syndrome	4 (10.8%)	6 (14.6%)	0.61
Previous MI	15 (40.5%)	12 (29.3%)	0.29
Previous PCI	16 (43.2%)	11 (26.8%)	0.12
Previous CABG	4 (10.8%)	10 (24.4%)	0.12
<b>Angina &lt;1 month CCS I-IV</b>	<b>18 (48.6%)</b>	<b>10 (24.3%)</b>	<b>0.03</b>
0 hour MDRD eGFR, ml/min, median(Q1-Q3)	51.2 (40.3-56.9)	47.5 (40-54.2)	0.24
0 hour Creatinine mg/dl, median (Q1-Q3)	123 (113-158)	126 (119-140)	0.59
eGFR 40-60 ml/min*	27 (72.9%)	29 (70.7%)	0.83
eGFR 20-40 ml/min*	10 (27.1%)	12 (29.3%)	0.83
eGFR <20 ml/min*	0	0	-
CCF, NYHA III-IV*	4 (10.8%)	5 (12.1%)	0.85
LV Ejection Fraction >50	17 (46.0%)	23 (56.1%)	0.37
LVEF 35-50	6 (16.2%)	5 (12.2%)	0.61
LVEF<35	2 (5.4%)	3 (7.3%)	0.73
Unknown LVEF	12 (32.4%)	10 (24.4%)	0.43
Haematocrit <0.39M/0.36Fs*	12 (32.4%)	18 (43.9%)	0.29
Blood Pressure, mmHg (SD)	132/76 (+/-19/10)	139/75 (+/-24/10)	0.16
Heart Rate, bpm (SD)	67	65	0.54
Contrast Volume ml (median/quartiles)	120 (81.5-200)	110 (90-156)	0.87
Hydration Volume, ml (SD)	750.29 (+/-169.1)	714.9 (+/-101.59)	0.26
Mehran Score <5 (SD)	11 (29.7%)	9 (21.9%)	0.19
Mehran Score 6-10 (SD)	18 (48.6%)	20 (48.7%)	0.99
Mehran Score 11-15 (SD)	6 (16.2%)	11 (26.8%)	0.26
Mehran Score >16 (SD)	2 (5.4%)	1 (2.4%)	0.49

\* CIN risk factors used as minimisation factors during randomisation

\*\* Independent t-test used for normally distributed variables, Man Whitney U test was used for non-parametric continuous variables and chi square test for categorical variables.

**Table 17: Baseline medication history**

	<b>Control (N=37)</b>	<b>RIPC (N=41)</b>	<b>P Value*</b>
Aspirin	33 (89.2%)	32 (78%)	0.18
<b>Clopidogrel/ P2Y(12) inhib</b>	<b>30 (81.1%)</b>	<b>25 (61%)</b>	<b>0.05</b>
B-Blocker	26 (70.3%)	33 (80.5%)	0.12
Ca Channel Blocker	12 (32.4%)	13 (31.7%)	0.94
<b>Nitrate</b>	<b>19 (51.4%)</b>	<b>9 (22%)</b>	<b>0.007</b>
Statin	33 (89.2%)	33 (80.5%)	0.29
ACE-I/ARB	23 (62.2%)	31 (75.6%)	0.20
Insulin	6 (16.2%)	6 (14.6%)	0.85
Sulphonylurea	7 (18.9%)	12 (29.3%)	0.28
Metformin	11 (29.7%)	12 (29.3%)	0.96
Glitazone	2 (5.4%)	1 (2.4%)	0.49
Gliptin	2 (5.4%)	1 (2.4%)	0.49
Warfarin	3 (8.1%)	7 (17.1%)	0.24
Diuretic	14 (37.8%)	17 (41.5%)	0.74
Nephrotoxic (NSAID etc.)	2 (5.4%)	1 (2.4%)	0.49

\* chi square test was used for categorical variables.

## 5.4 SERUM CREATININE RESULTS

Table 18: Serum creatinine values at 0 hours, 48 hours and 3 months

Creatinine ( $\mu\text{mol/l}$ )	Control Median (Q1-3) n= 37	RIPC Median (Q1-3) n= 41
0 hour	123 (113-157)	126 (118.5 – 140)
48 Hour	133 (105 -153)	128 (114 – 152)
	Control n= 35	RIPC n= 38
3 Month Median	126 (114-150)	124.5 (106 – 147)

Figure 20: Serum creatinine values at 0 hours, 48 hours and 3 months

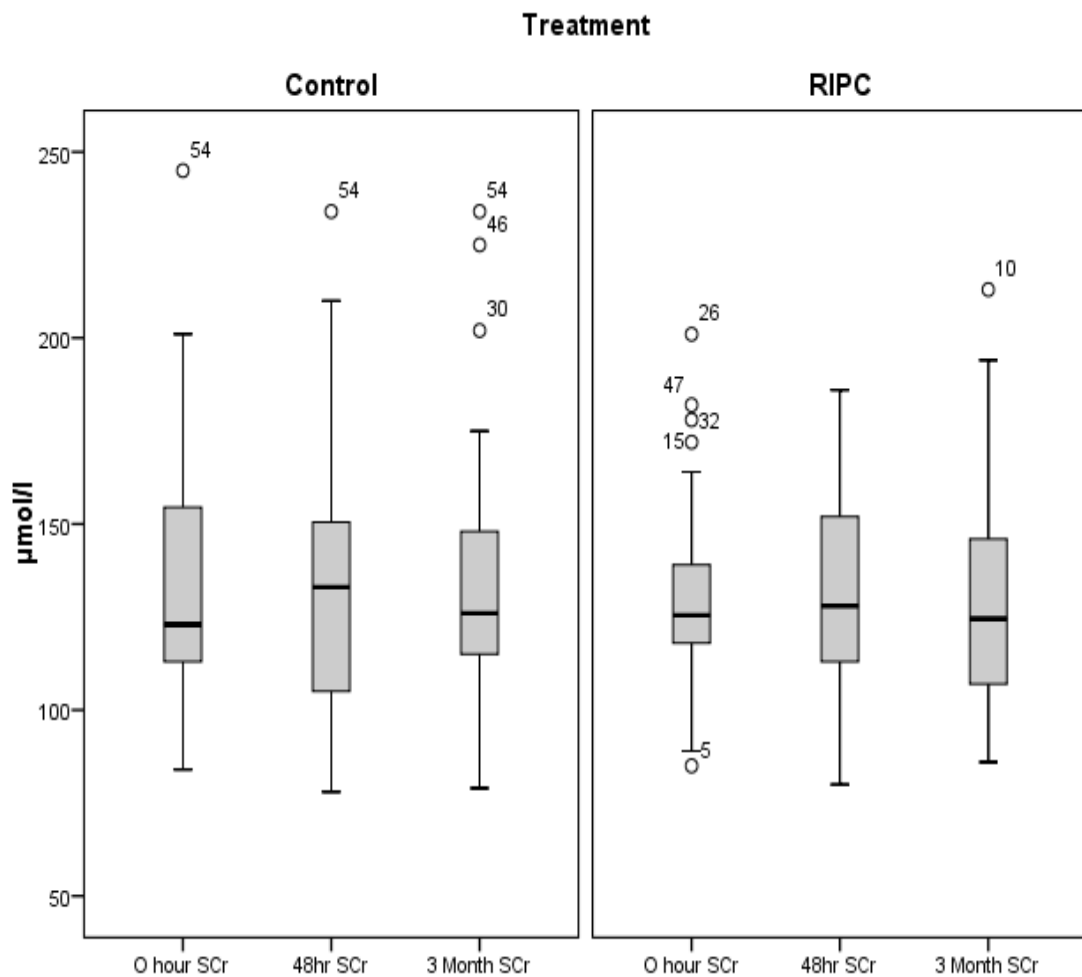




Figure 21: Individual participant serum creatinine change at 48 hours

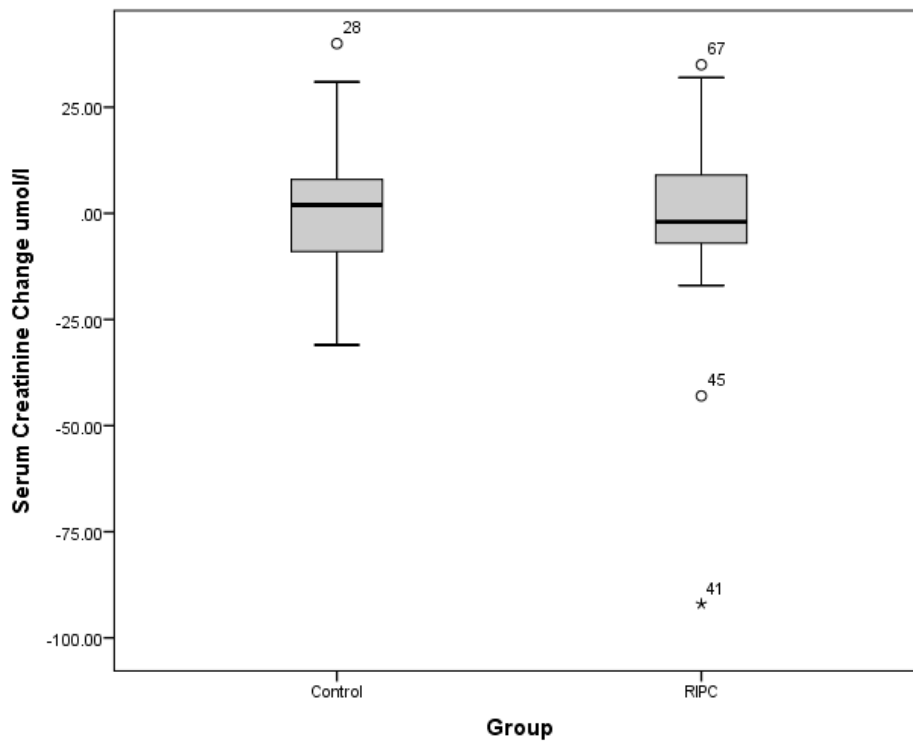
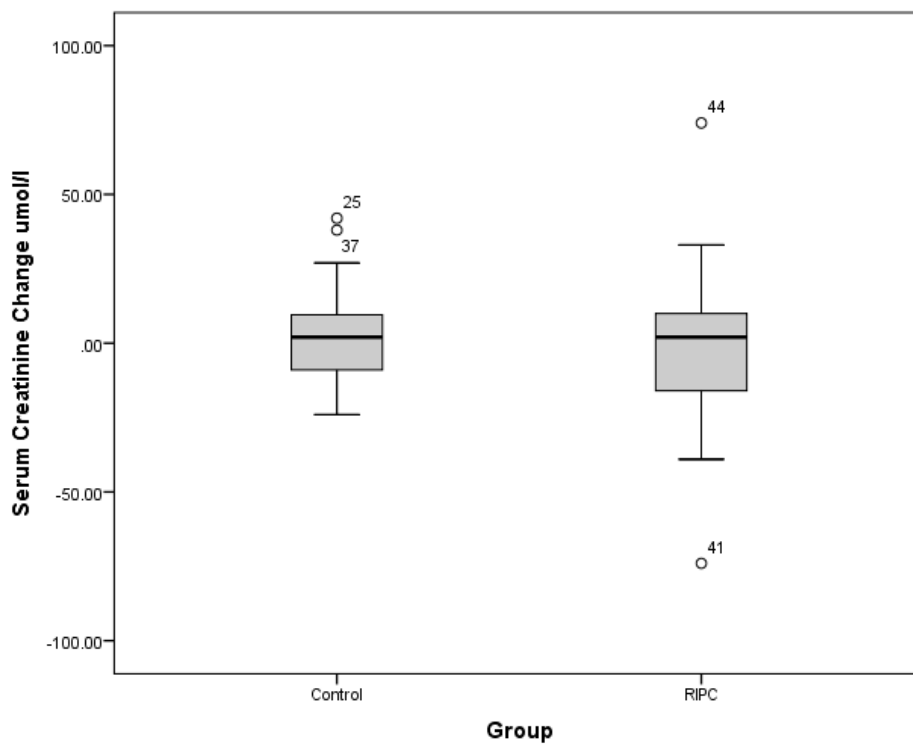


Figure 22: Individual participant serum creatinine change at 3 months



## 5.5 EGFR RESULTS

Table 19: eGFR values at 0 hours, 48 hours and 3 months

eGFR ml/min	Control n= 37	RIPC n= 41
0 hour Median (Q1-3)	51.2 (40.3 – 56.9)	47.5 (40.1-54.2)
48 Hour Median (Q1-3)	48.7 (41.2 – 58.6)	46.8 (39.8 – 56.46)
	Control n= 35	RIPC n= 38
3 Month Median (Q1-3)*	47.13 (39.9 – 56.6)	47.8 (37.8- 587.5)

Figure 23: eGFR values at 0 hours, 48 hours and 3 months

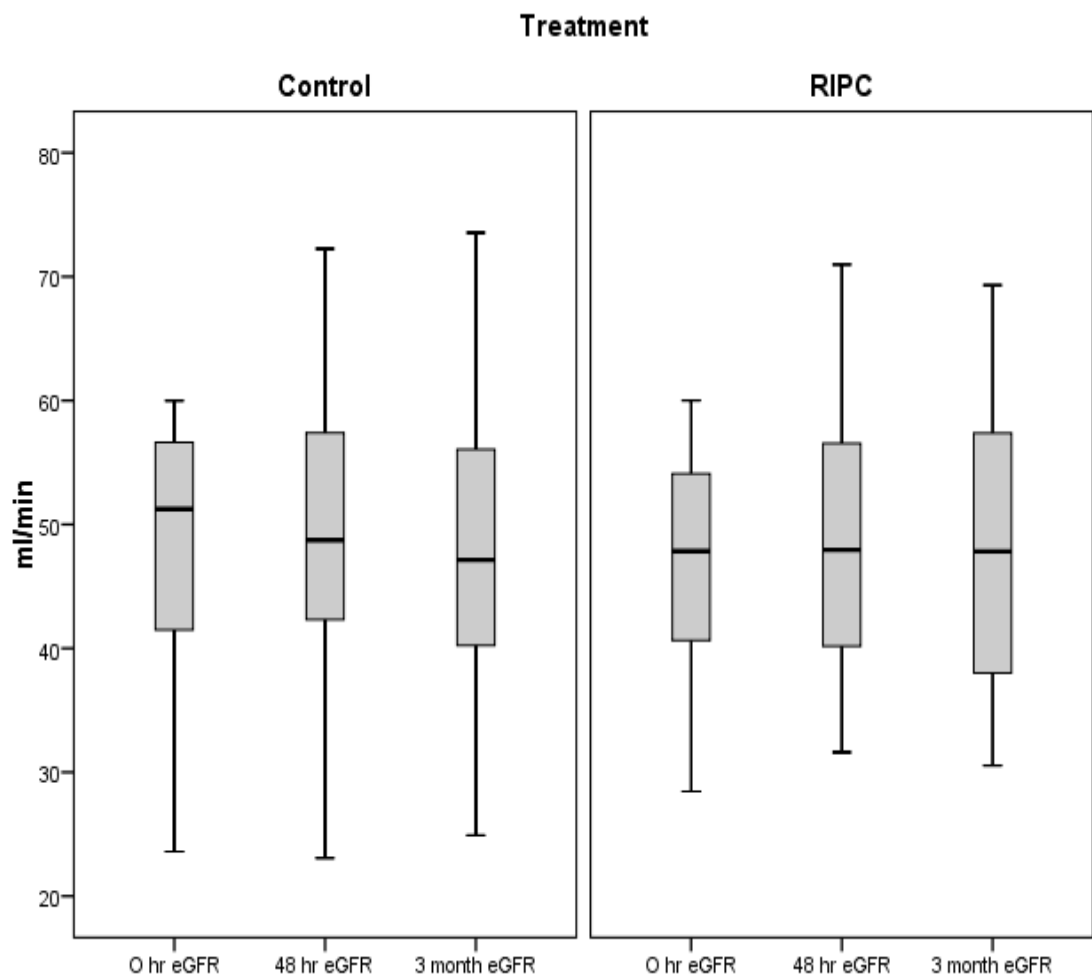


Figure 24: Individual participant eGFR change from 0 to 48 hours

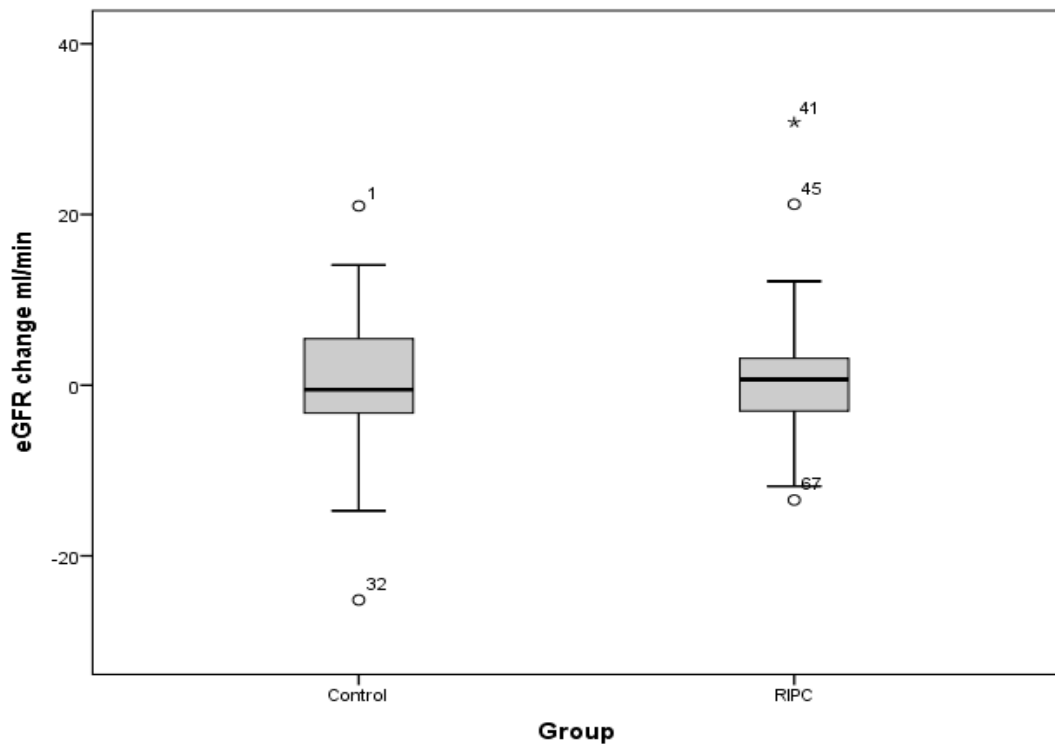
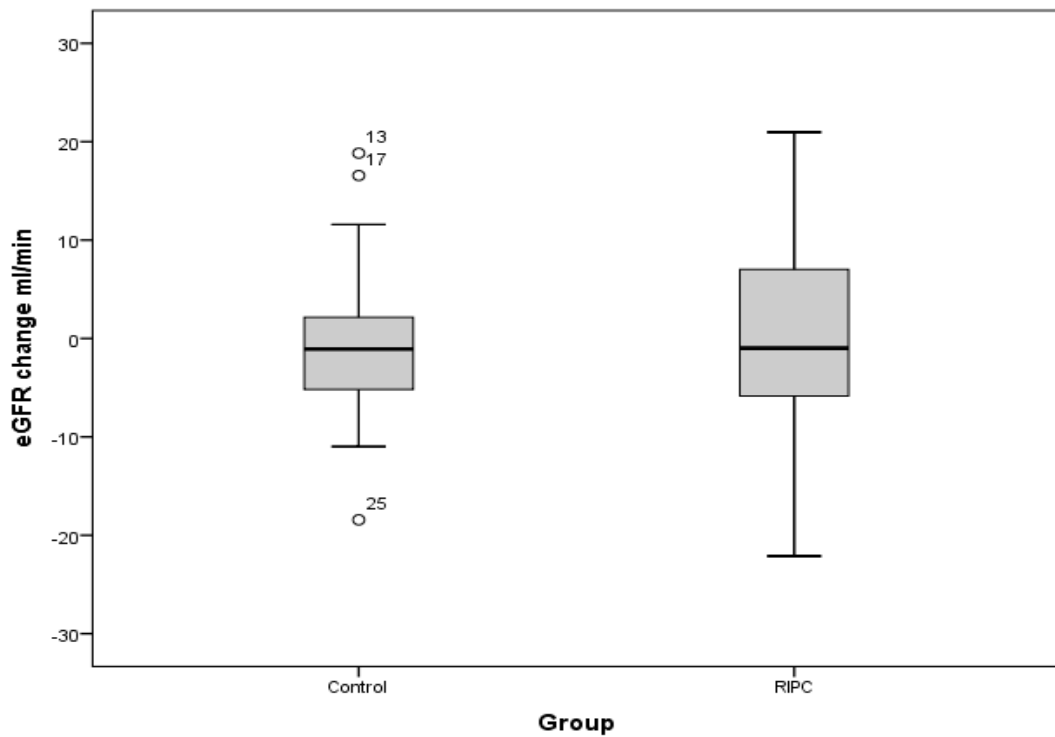


Figure 25: Individual participant eGFR change from 0 hour to 3 months



## 5.6 URINE ALBUMIN CREATININE RATIO RESULTS

Table 20: UACR values at 0 hours, 48 hours and 3 months

Urine Albumin Creatinine Ratio mg/mmol	Control n= 32	RIPC n= 37
0 hour Median (Q1-3)	2.1 (0.5 -9.1)	1.6 (0.4 – 5.8)
48 Hour Median (Q1-3)	1.8(0.6 – 13.1)	1.7 (0.5- 4.9)
	Control n= 25	RIPC n=28
3 Month Median (Q1-3)	1.7 (0.2 – 11.7)	1.3 ( 0.7 - 4.2)

Figure 26: UACR values at 0 hours, 48 hours and 3 months

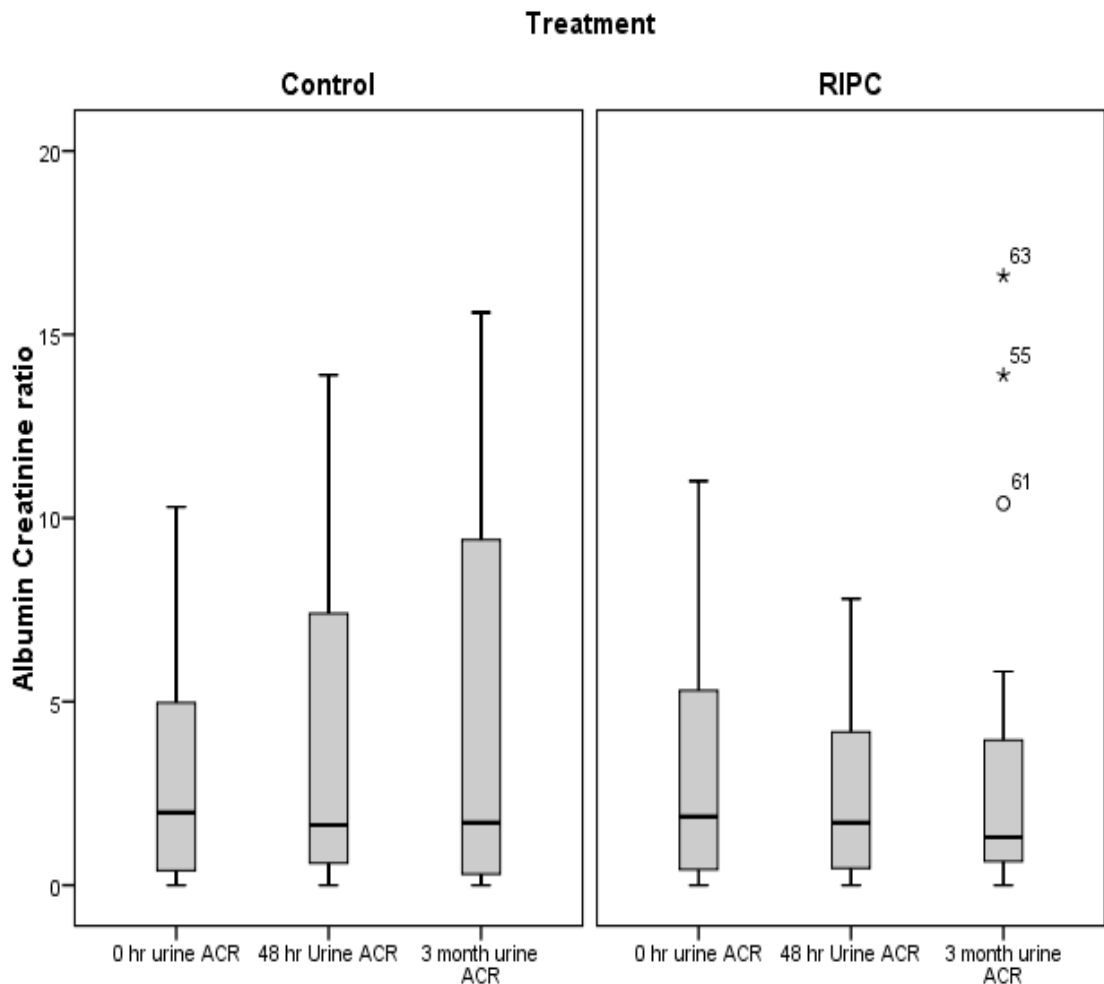


Figure 27: Individual participant UACR change from 0 to 48 hours

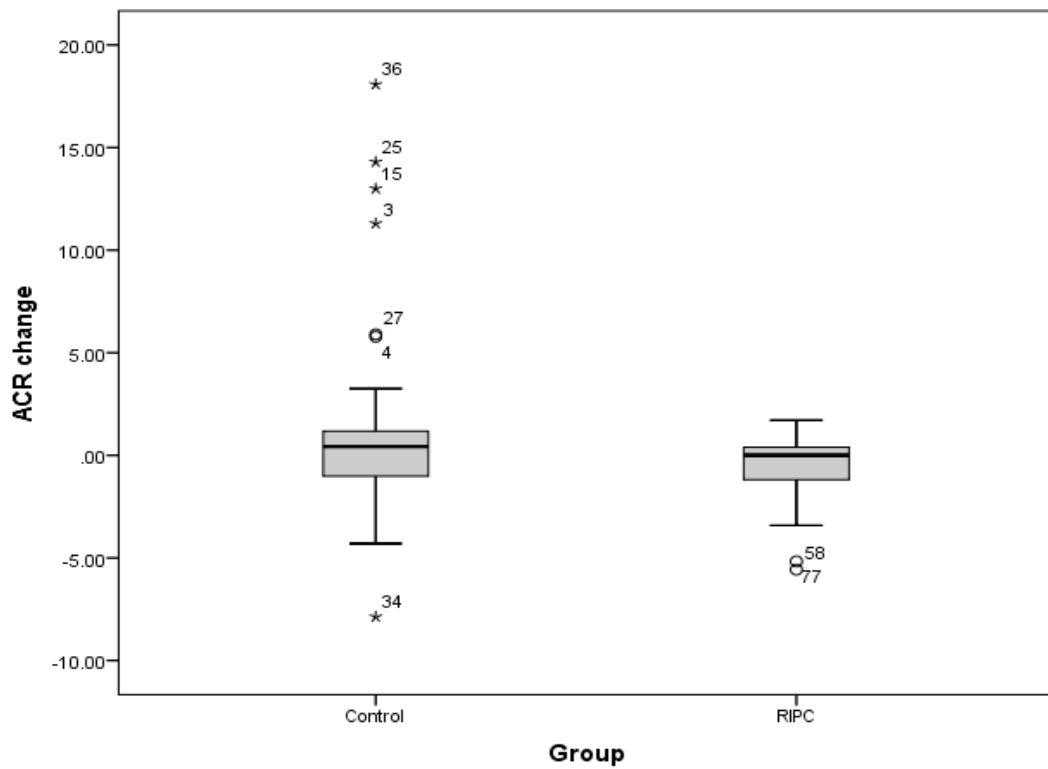
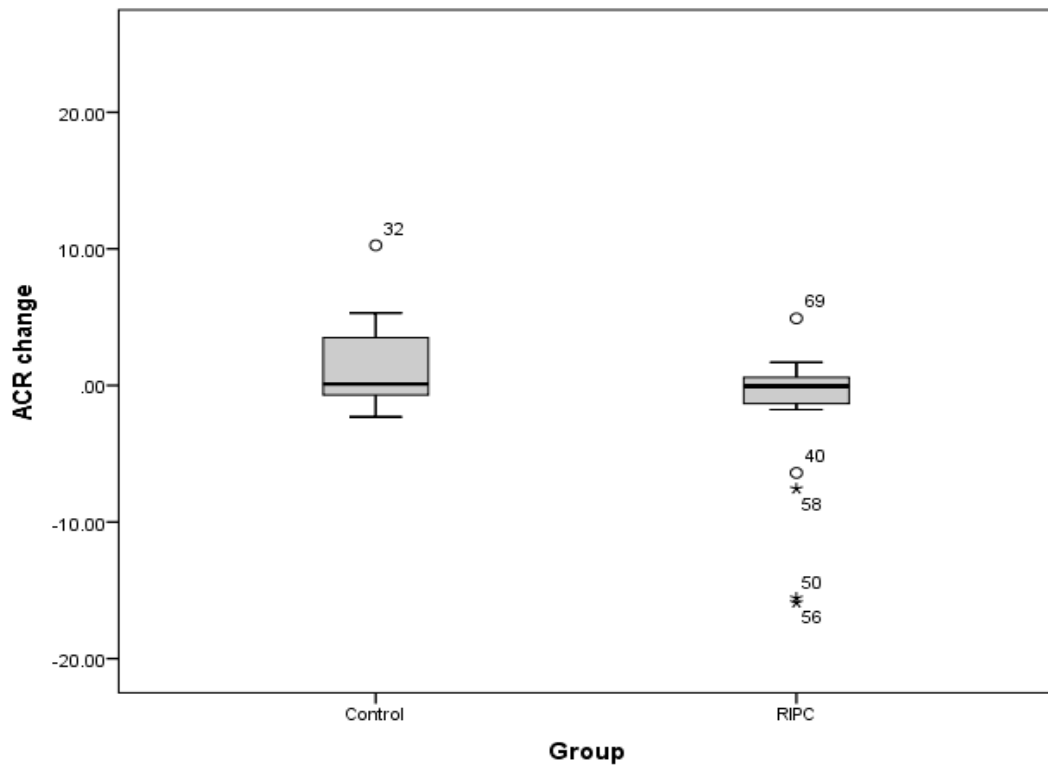


Figure 28: Individual participant UACR change from 0 hour to 3 months

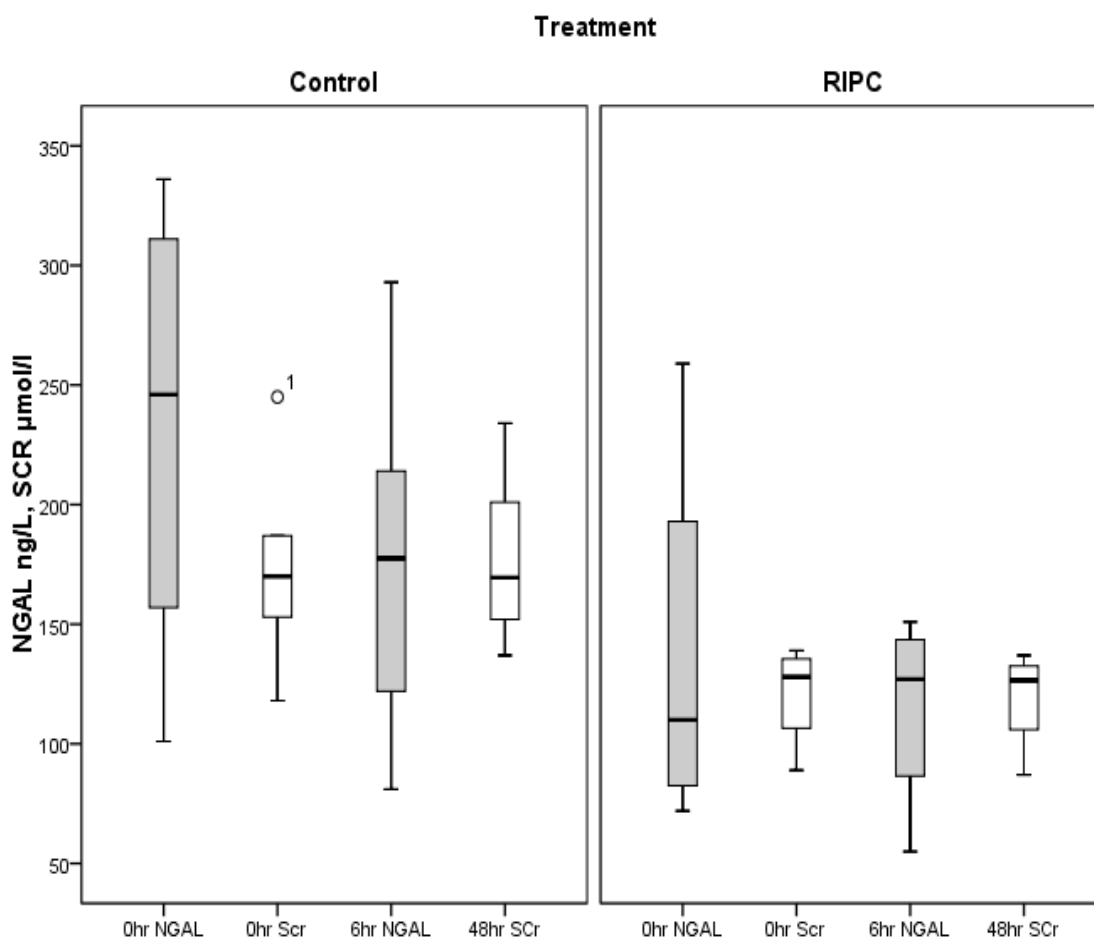


## 5.7 'POINT OF CARE' NGAL RESULTS

Table 21: NGAL values at 0 and 6 hours and creatinine at 0 and 48 hours

NGAL (ng/l)	Control Median (Q1-3) n= 6	RIPC Median (Q1-3) n= 4
0 hour	246 (143 to 317.5)	110 (77.3 to 226.0)
6 hours	177.5 (111.8 to 233.8)	127 (70.8 to 147.3)
SCr ( $\mu\text{mol/l}$ )		
0 hour	170 (144.3 to 201.5)	128.0 ( 97.8 to 137.3)
48 hours	169 (97.7 to 137.2)	127 (96.5 to 134.8)

Figure 29: NGAL and SCr values at 0 and 6 hours and SCr at 0 and 48 hours



## 5.8 PRIMARY AND SECONDARY OUTCOME MEASURES

Table 22: Primary and Secondary outcome measure analysis

Primary Outcome	Control Group (n=37)	RIPC Group (n=41)	Odds Ratio & Significance
<b>CIN</b>  (25% or 44 $\mu\text{mol/l}$ increase in Creatinine)  n, (%)	2 (5.4%)	2 (4.8%)	<b>OR 1.1</b> (CI 0.15 to 8.33) <b>p = 0.916</b>  <b>Adj. OR 1.9 *</b> (CI 0.19 to 20.5) <b>p = 0.575</b>
Secondary Outcomes	Control Group Median (Q1-Q3)	RIPC Group Median (Q1-Q3)	Significance**
0-48 hour SCr $\Delta$ $\mu\text{mol/l}$	1 (-13.5 to 13.5)	0.5 (-6.8 to 10.5)	<b>p = 0.97</b>
0-48 hour eGFR $\Delta$ ml/min	0.9 (-4.3 to 8.4)	-0.3 (-3.4 to 3.3)	<b>p = 0.834</b>
0-48 hour UACR $\Delta$ mg/mmol	0.7 (-0.2 to 4.5)	0 (-1.3 to 0.3)	<b>p = 0.09</b>
0-3 month SCr $\Delta$ , $\mu\text{mol/l}$	2.0 (-12.0 to 9.5)	2.0 (-14.3 to 9.8)	<b>p = 0.703</b>
0-3 month eGFR $\Delta$ , ml/min	-1.1 (-5.2 to 2.2)	-1.1 (-5.6 to 8.5)	<b>p = 0.703</b>
0-3 month UACR $\Delta$ mg/mmol	0.1 (-0.9 to 3.7)	0.0 (-1.4 to 0.6)	<b>p = 0.206</b>
0-6 hour NGAL $\Delta$ ng/l	-59.5 (-98.7 to -19.5)	-4.0 (-85.2 to 21)	<b>p = 0.394</b>
Secondary Outcome	Control Group (n=37)	RIPC Group (n=41)	Odds Ratio & Significance
<b>Cardio-renal Endpoints</b>  n, (%)	<b>3 (8.1%)</b>  1 Death (Non-CV) 1 ACS 1 Readmission with haemorrhage	<b>2 (4.8%)</b>  1 Haemorrhage 1 Acute LVF	<b>OR 1.7</b> (CI 0.27 –to 16.8) <b>p=0.565</b>  <b>Adj. OR 2.2*</b> (CI 0.29 to 18.2) <b>p = 0.437</b>

\* Multivariate regression analysis adjusted for variables including Age>75, eGFR (ranges: <20, 20-40, 40-60), diabetes, CCF or LVEF<50%, anaemia, hypotension and contrast volume.

\*\* Mann-Whitney U-test used for non-parametric analysis

## 5.9 SUBGROUP ANALYSIS

Table 23: Subgroup analysis of patients aged > 75

AGE>75 n=28	Control Group Med(Q1-Q3) n = 12	RIPC Group Med(Q1-Q3) n= 16	Significance**
0-48 hour SCr $\Delta$ $\mu\text{mol/l}$	5.0 (-3.5 to 23.2)	1.5 (-6.8 to 14.0)	p = 0.285
0-48 hour eGFR $\Delta$ ml/min	-2.0 (-11.2 to 1.2)	-1.2 (-5.2 to 2.3)	p = 0.246
0-48 hour UACR $\Delta$ mg/mmol	-4.1 (-2.1 to 8.0)	0.23 (-3.2 to 1.0)	p = 0.535
0-3 month SCr $\Delta$ $\mu\text{mol/l}$	3.0 (-8.0 to 22.0)	-2.0 (-21 to 18.0)	p = 0.232
0-3 month eGFR $\Delta$ ml/min	-5.5 (-9.6 to -0.4)	1.03 (-6.4 to 8.9)	p = 0.082
0-3 month UACR $\Delta$ mg/mmol	-1.6 (-35.8 to 1.0)	-0.53 (-15.6 to 0.3)	p = 0.643

\*\* Mann-Whitney U-test used for non-parametric analysis

Table 24: Subgroup analysis of patients with an eGFR<40ml/min

eGFR<40ml/min n=22	Control Group Med(Q1-Q3) n = 10	RIPC Group Med(Q1-Q3) n=12	Significance**
0-48 hour SCr $\Delta$ $\mu\text{mol/l}$	2.5 (-13.5 to 10.3)	-3.5 (-6.75 to 11.8)	p = 0.792
0-48 hour eGFR $\Delta$ ml/min	1.96 (-2.9 to 4.9)	0.99 (-2.64 to 2.2)	p = 0.742
0-48 hour UACR $\Delta$ mg/mmol	0.61 (-1.9 to 10.5)	0.19 (-1.47 to 1.3)	p = 0.657
0-3 month SCr $\Delta$ $\mu\text{mol/l}$	-10 (-15.5 to 1.5)	7 (-31.5 to 13.2)	p = 0.744
0-3 month eGFR $\Delta$ ml/min	1.28 (-5.9 to 3.8)	-1.65 (-4.9 to 12.5)	p = 0.624
0-3 month UACR $\Delta$ mg/mmol	1.61 (-25.5 to 13.2)	-0.37 (-1.3 to 0.4)	p = 0.631

\*\* Mann-Whitney U-test used for non-parametric analysis

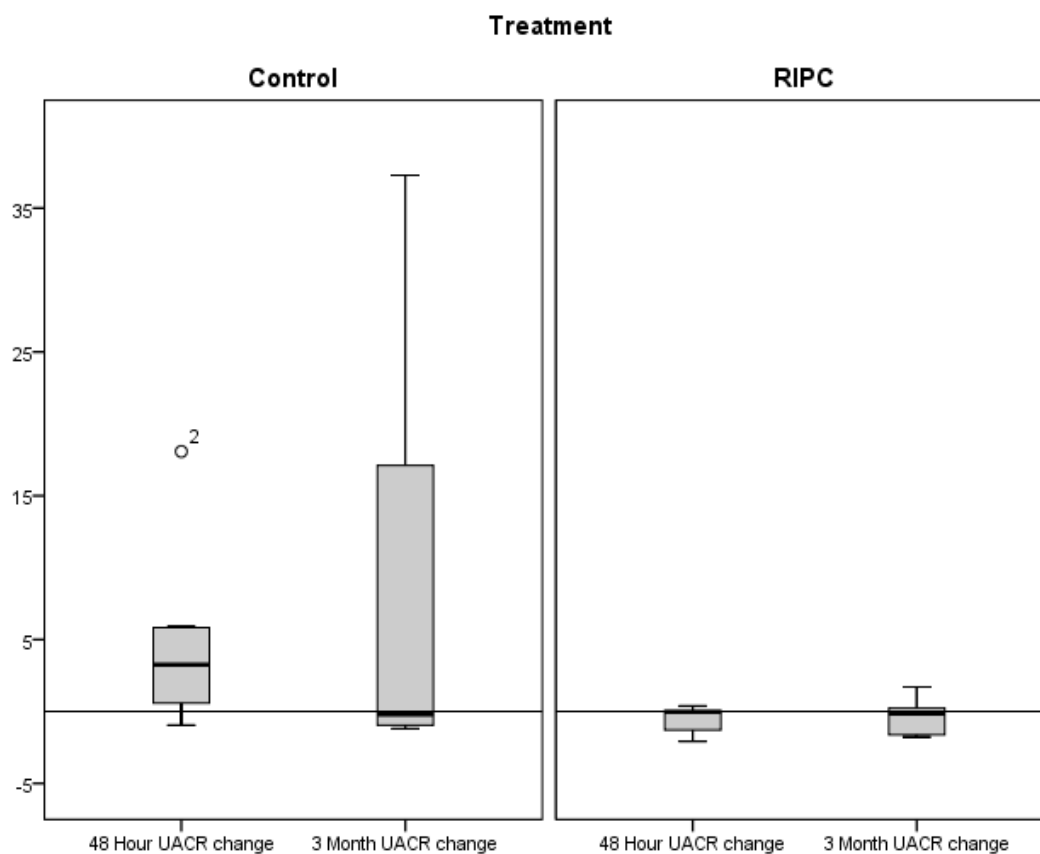


**Table 25: Subgroup analysis of patients with CCF or an LVEF<50%**

<b>CCF or LVEF&lt;50%</b> n=20	<b>Control Group</b> Med(Q1-Q3) n= 9	<b>RIPC Group</b> Med(Q1-Q3) n= 11	<b>Significance**</b>
<b>0-48 hour SCr Δ</b> μmol/l	-7.0 (-10.0 to 6.5)	-4.0 (-7 to 5.0)	<b>p = 0.648</b>
<b>0-48 hour eGFR Δ</b> ml/min	2.6 (-1.8 to 8.9)	1.3 (-1.3 to 3.8)	<b>p = 0.676</b>
<b>0-48 hour UACR Δ</b> mg/mmol	<b>2.1 (0.4 to 5.9)</b>	<b>0.0 (-1.3 to 0.3)</b>	<b>p = 0.009</b>
<b>0-3 month SCr Δ</b> μmol/l	-3.0 (-15.0 to 2.0)	-4.0 (-22.5 to 8.0)	<b>p = 0.682</b>
<b>0-3 month eGFR Δ</b> ml/min	-0.2 (-1.1 to 7.1)	1.5 (-6.3 to 10.3)	<b>p = 0.870</b>
<b>0-3 month UACR Δ</b> mg/mmol	-0.2 (-1.2 to 30.8)	-0.15 (-1.8 to 0.5)	<b>p = 0.482</b>

\*\* Mann-Whitney U-test used for non-parametric analysis

**Figure 30: UACR change at 48 hours and 3 months in CCF or LVEF<50%**  
(No CIN occurred in either group)



**Table 26: Subgroup analysis of patients with type 2 diabetes**

Type 2 Diabetes n=37	Control Group Med(Q1-Q3) n =18	RIPC Group Med(Q1-Q3) n =19	Significance**
0-48 hour SCr $\Delta$ $\mu\text{mol/l}$	4.0 (-15.6 to 10.3)	3.0 (-4.0 to 14.5)	<b>p = 0.484</b>
0-48 hour eGFR $\Delta$ ml/min	-2.32 (-6.2 to 9.7)	-1.45 (-4.0 to 1.6)	<b>p = 0.584</b>
0-48 hour UACR $\Delta$ mg/mmol	0.87 (-1.5 to 13.5)	0.1 (-1.63 to 1.1)	<b>p = 0.265</b>
0-3 month SCr $\Delta$ $\mu\text{mol/l}$	1.0 (-11.5 to 10.5)	9.0 (-7.0 to 19.5)	<b>p = 0.188</b>
0-3 month eGFR $\Delta$ ml/min	-3.1 (-5.1 to 3.8)	-4.55 (-7.6 to 3.4)	<b>p = 0.222</b>
0-3 month UACR $\Delta$ mg/mmol	2.4 (0.5 to 11.6)	-.05 (-3.2 to 1.3)	<b>p = 0.095</b>

\*\* Mann-Whitney U-test used for non-parametric analysis

**Table 27: Subgroup analysis of patients with anaemia**

Anaemia n=24	Control Group Med(Q1-Q3) n =12	RIPC Group Med(Q1-Q3) n =18	Significance**
0-48 hour SCr $\Delta$ $\mu\text{mol/l}$	0.0 (-10.0 to 17.0)	0.5 (-6.0 to 14.0)	<b>p = 0.966</b>
0-48 hour eGFR $\Delta$ ml/min	1.10 (-7.6 to 4.4)	-0.39 (-4.4 to 2.8)	<b>p = 0.641</b>
0-48 hour UACR $\Delta$ mg/mmol	0.52 (-0.78 to 11.5)	0.0 (-4.73 to 0.4)	<b>p = 0.066</b>
0-3 month SCr $\Delta$ $\mu\text{mol/l}$	-1.0 (-10.8 to 14.3)	-0.5 (-18.0 to 13.8)	<b>p = 0.672</b>
0-3 month eGFR $\Delta$ ml/min	0.6 (-8.6 to 3.0)	0.2 (-5.9 to 7.5)	<b>p = 0.611</b>
0-3 month UACR $\Delta$ mg/mmol	-0.2 (-47 to 3.9)	-0.5 (-15.6 to 1.2)	<b>p = 0.928</b>

\*\* Mann-Whitney U-test used for non-parametric analysis

**Table 28: Subgroup analysis of patients, Mehran score >6 (Moderate CIN risk)**

<b>Mehran score &gt;6 n=58</b>	<b>Control Group Med(Q1-Q3) n =26</b>	<b>RIPC Group Med(Q1-Q3) n =32</b>	<b>Significance**</b>
<b>0-48 hour SCr Δ μmol/l</b>	4.5 (-8.0 to 18.0)	2.5 (-6.0 to 13.2)	<b>p = 0.827</b>
<b>0-48 hour eGFR Δ ml/min</b>	-0.6 (-6.2 to 4.9)	-1.1 (-3.8 to 2.1)	<b>p = 0.737</b>
<b>0-48 hour UACR Δ mg/mmol</b>	0.7 (-1.2 to 5.8)	0.0 (-1.8 to 0.7)	<b>p = 0.059</b>
<b>0-3 month SCr Δ μmol/l</b>	1.0 (-12.0 to 8.5)	5.0 (-17.8 to 14.8)	<b>p = 0.886</b>
<b>0-3 month eGFR Δ ml/min</b>	-1.1 (-5.9 to 2.9)	-1.7 (-6.8 to 7.5)	<b>p = 0.813</b>
<b>0-3 month UACR Δ mg/mmol</b>	0.2 (-1.2 to 4.0)	-0.3 (-1.6 to 0.9)	<b>p = 0.261</b>

\*\* Mann-Whitney U-test used for non-parametric analysis

**Table 29: Subgroup analysis of patients, Mehran score>11 (High CIN risk)**

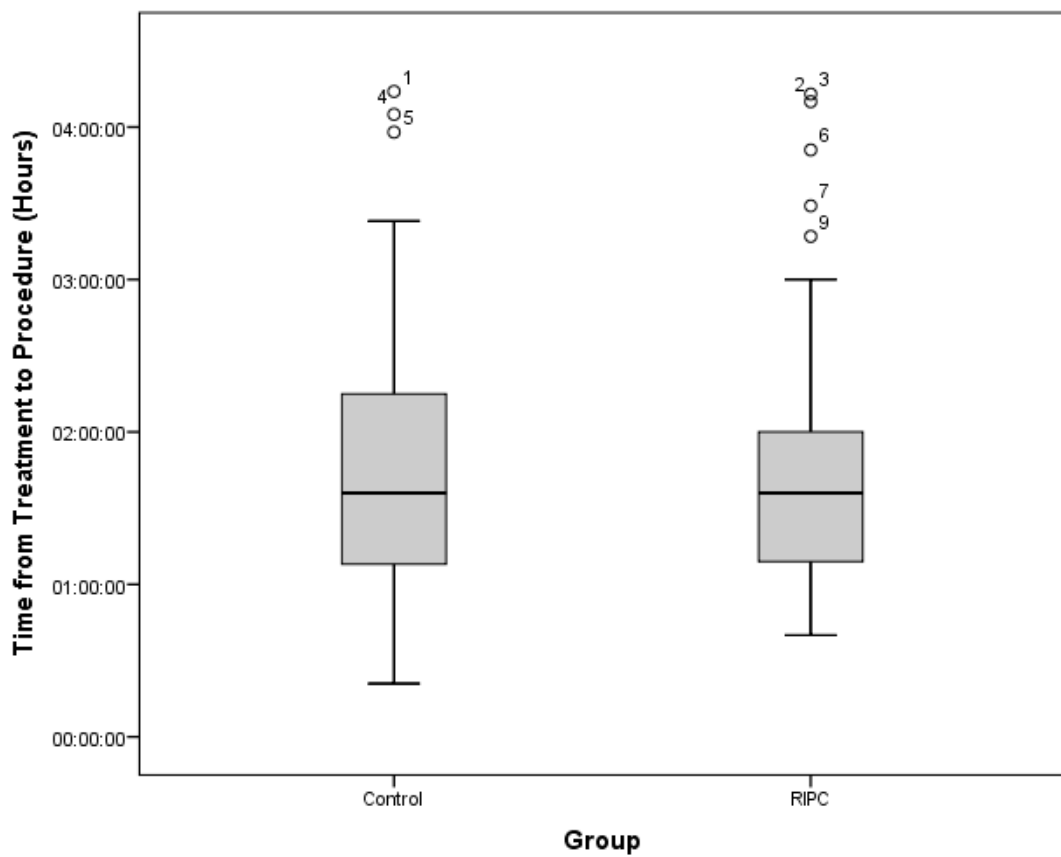
<b>Mehran score&gt;11 n=20</b>	<b>Control Group Med(Q1-Q3) n =8</b>	<b>RIPC Group Med(Q1-Q3) n =12</b>	<b>Significance**</b>
<b>0-48 hour SCr Δ μmol/l</b>	5.0 (-3.5 to 22.3)	4.5 (-3.75 to 14.0)	<b>p = 0.670</b>
<b>0-48 hour eGFR Δ ml/min</b>	-3.7 (-11.2 to 0.8)	-1.2 (-6.3 to 2.1)	<b>p = 0.247</b>
<b>0-48 hour UACR Δ mg/mmol</b>	2.0 (-1.6 to 15.2)	0.4 (-2.6 to 1.2)	<b>p = 0.195</b>
<b>0-3 month SCr Δ μmol/l</b>	1.5 (-6.8 to 34.0)	8.0 (-17.0 to 19.0)	<b>p = 0.710</b>
<b>0-3 month eGFR Δ ml/min</b>	1.1 (-4.0 to 20.1)	-2.1 (-6.7 to 8.9)	<b>p = 0.283</b>
<b>0-3 month UACR Δ mg/mmol</b>	-2.0 (-70.9 to 19.3)	0.0 (-8.7 to 1.0)	<b>p = 0.602</b>

\*\* Mann-Whitney U-test used for non-parametric analysis

## 5.10 QUALITY ASSURANCE OF RIPC THERAPY

Time from Sham/RIPC to contrast exposure	Control n= 37	RIPC n= 41
Hour : min (SD)	1:46 (0:57)	1:49 (0:55)

Figure 31: Time from sham/RIPC treatment to contrast exposure



100% of all Sham/RIPC treatments were delivered as per protocol (4 cycles of 5 minute inflation to 10mmHg/200mmHg followed by 5 minute deflation) as recorded in the DERIC device software log.

## 5.11 SIGNIFICANT ADVERSE EVENTS

No SAE's were recorded relating to blood pressure cuff inflation in either group

### **Control group**

1. One patient without CIN was readmitted to hospital with suspected ACS with no ECG changes, negative Troponin T and no acute obstructive lesion on repeat coronary angiography.
2. One patient without CIN died during follow up due to complications during elective cancer surgery
3. One patient without CIN was readmitted to hospital during follow up with an upper gastrointestinal bleed requiring blood transfusion.

### **RIPC group**

1. One patient suffered a femoral haematoma and hypotension post procedure and developed CIN
2. One patient without CIN was readmitted to hospital with decompensated heart failure during follow up.
3. One patient developed an allergic contrast reaction and required oral steroids but did not develop CIN.

## **5.12 PATIENT FOCUS GROUP RESPONSES**

Audio recordings from the two separate open question and answer sessions involving 6 control group participants and 6 RIPC group participants were analysed and summarised.

### **A) Initial contact with research team and Information sheet**

The majority of patients (67% in both groups) recalled being initially approached by a member the research team in person on the day of their procedure, with 33% Control, 17% RIPC recalling the introductory phone call with a member of the research team, and 17% of the RIPC group unsure. All participants were satisfied with the initial contact although 33% in both groups wished they had been approached earlier. 50% of patients in both groups felt anxiety about their cardiac procedure made understanding the PIS more difficult although no participants reported that they were placed under undue pressure to participate in the study. 100% of patients in both groups understood the research activities and follow up process and were given contact details of the research team. 50% of patients in both groups learned about their renal dysfunction for the first time during the initial contact and all felt they had been adequately counselled about this finding.

### **B) On the day of the procedure**

100% of all participants felt that the research procedure had been adequately explained to them and found the research team to be polite and helpful. No patients reported adverse effects from the RIPC/Sham procedure and none felt that the research procedures had interfered with their usual care. RIPC patients reported only mild discomfort from the cuff inflation, in one example a participant reported “you get used to it”.

### **C) Follow up after your procedure**

100% of participants reported receiving adequate instructions about where and when the blood tests were to be collected. However 17% of patients in both groups attended the wrong location for blood tests although this was quickly rectified after contacting the research team. 33% of participants in both groups attended satellite hospitals for blood sample collection and found this process to be straightforward and convenient. 33% of participants in both groups would have liked to attend their GP for follow up blood tests although recognised that this may have been difficult due to high GP workload.

### **D) Open feedback session**

83% of patients in both groups expressed interest in receiving feedback from the outcome of study in the form of a newsletter, with 17% expressing interest in feedback via a website. 67% of patients in both groups wished they had been approached about the study at the initial Coronary Angiography pre-assessment clinic. The challenge of identifying suitable participants, many of whom would have an unknown eGFR prior to pre-assessment was discussed with participants in both groups and early involvement of GP's was recognised as a potential solution.

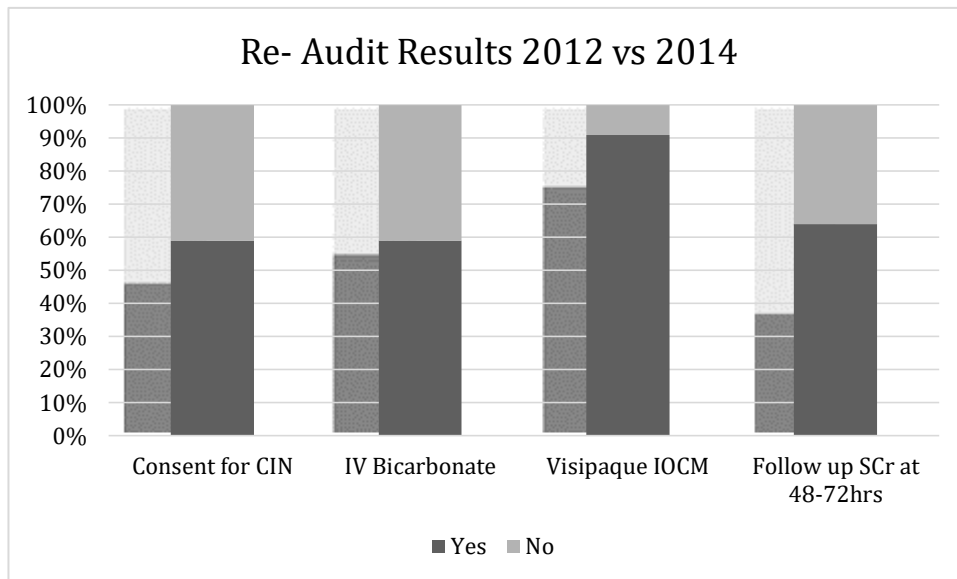
## CHAPTER 6: CIN RE-AUDIT

In order to close the audit loop, a re-audit of patients at undergoing CA or PCI at UCLH/The Heart Hospital was undertaken over a 3-month period from 1st March – 31st May 2014, identifying a total of 614 patients.

- The CDR database was used to determine the eGFR and creatinine prior to the procedure and, where available, at 48-72 hours and at 3 months afterwards
- Further blood results were retrieved from the TOMCAT database where data was not available on CDR (i.e. inter-hospital “treat and returns”)
- Clinical records were ordered to determine if:
  - i.v. sodium bicarbonate had been prescribed and administered
  - Appropriate consent to include CIN had been obtained
  - A repeat (48-72h) blood test had been requested
  - The new WHO check list had been carried out and the form completed
- Mean age was 63; 68% were male
- 15% were “treat and returns”
- 83% had no follow-up bloods within UCLH NHS trust
- 16% (100 patients) had an eGFR<60 ml/min
- Rate of CIN in this high-risk cohort: was 6-14%
- 30-day mortality rate was 1.95% (no significant difference from 2012)
- A WHO check list was present in 27% of case notes analysed



Figure 32: UCLH CIN audit 2012 compared with re-audit 2014



## **CHAPTER 7: DISCUSSION**

### **7.1 CLINICAL AUDIT**

The most striking finding to emerge from the Clinical Audit in 2012 was that in patients undergoing CA or PCI at UCLH, those with at least moderate pre-existing renal impairment had a five-fold increase in mortality at 30 days compared to patients with normal renal function, with the development of CIN leading to a further doubling mortality in this high risk group. The onset of CIN may not have played a causal role in all cases, perhaps acting as a marker of severe multi-organ injury, although an extensive body of evidence clearly demonstrates that CIN is independently associated with poor cardio-renal outcomes (section 1.3.3). The principle finding of the Audit was that compliance with the UCLH CIN prevention protocol was markedly suboptimal, which catalysed conception and design of the clinical trial in tandem with a reinvigorated educational programme and audit cycle, in order to improve standards of care and patient outcomes.

### **7.2 ERICCIN OUTCOMES**

In this multicentre double blinded randomised controlled trial, enrolling 100 patients at risk of CIN undergoing CA or PCI, standard CIN prophylaxis plus RIPC (4 cycles of 5 minute upper limb ischaemia and reperfusion) did not reduce the incidence of CIN at 48 hours (5.4% vs 4.8%, OR 1.1 (CI 0.15 to 8.33),  $p = 0.91$ ) even when adjusted for the main CIN risk factors of age >75, eGFR, diabetes, CCF, anaemia, hypotension and contrast media volume (Adjusted OR 1.9, CI 0.19 to 20.5,  $p = 0.575$ ). There were no significant differences in SCr, eGFR or UACR at 48 hour or 3 months between control and RIPC groups and no effect was observed on cardio-renal endpoints.

A small but non-significant trend towards abrogation of median SCr increase at 48 hours after RIPC was seen. No significant change in SCr occurred between pre-angiography and 3 month sample points within either group, which is not surprising given the low incidence of CIN and minimal deterioration in renal function expected in CKD over the short course of the study.

Subgroup analysis of patients with CCF receiving RIPC demonstrated a small but significant abrogation in UACR increase at 48 hours (2.1 vs 0 mg/mmol,  $Z = -2.598$ ,  $r = 0.63$ ,  $p=0.009$ ), which did not persist at 3 months. This was observed in the context of a general but non-significant trend towards abrogation in UACR increase at 48 hours in all patients treated with RIPC, particularly in those with anaemia or Mehran scores  $>6$ . However it is important to recognise that these findings are significantly underpowered and cannot establish a conclusive relationship, but may provide a hypothesis-forming basis for future CIN studies.

### **7.2.1 LOWER THAN EXPECTED CIN INCIDENCE**

In trial enrolled patients, where CIN prophylaxis was 100% compliant with pre-hydration, Visipaque use and minimised administered contrast volumes (mean 120ml, SD 88.5-200ml) a precipitous reduction in the incidence of CIN(5%) was observed. Due to the much lower than expected incidence of CIN at interim data analysis, the DMC for the study deemed that the trial was significantly underpowered and recommended halting further recruitment on grounds of futility. With a CIN incidence of approximately 5% and an assumed RIPC effect of a 60% reduction in CIN, 1176 participants would have been required to achieve a power of 80% and an  $\alpha$  value of 0.05. This was felt to be well beyond the scope of a proof of concept investigation, although might be achievable in a larger clinical trial should a supportive body of evidence accumulate from similar pilot studies.

Changes in serum NGAL were not assessed due to resource constraints in the context of a neutral study outcome, with the DMC recommendation that NGAL analysis was unlikely to yield further useful information. Point of care NGAL could not be fully assessed due to the small number of tests performed, with the sole intention at the outset to correlate a small panel of these results with laboratory NGAL samples at the close of the study.

## **7.3 POTENTIAL CAUSES FOR LOW CIN INCIDENCE**

### **7.3.1 FEASIBILITY ERROR**

The initial CIN audit in 2012 suggested that a CIN rate of 15% was to be expected in the control group, however a much lower incidence of approximately 5% CIN was observed. This disparity may have been attributable to sampling bias in the audit, leading to overestimation of the true CIN incidence. Only 37% of patients at risk of CIN subsequently had 48-hour SCr results documented at UCLH, with the vast majority of this cohort requiring a prolonged inpatient stay following their procedure, potentially due to procedural complications, severity of illness or comorbidity and thus were more likely to have developed CIN. Of the remaining 63% at-risk patients with no documented 48 hour creatinine results at UCLH, most were referred to their GP or referring hospitals for follow up, and in some repeat SCr may not have occurred within 72 hours. However a lower rate of CIN is more likely in this group when compared to inpatient group, although to what extent it is difficult to quantify and inclusion of the missing GP data was beyond the scope of the initial audit. As a result the initial power calculation for the study is likely to have underestimated the recruitment target that was necessary to achieve appropriate power.

### 7.3.2 PARTICIPANT SELECTION

A degree of selection bias may have occurred favouring inclusion of lower risk patients, as higher risk patients were more likely to decline participation, be subsequently excluded, or experience difficulties attending follow up at the nominated time points. Of the 244 patients eligible for inclusion to the study, over 46% declined participation at the outset, most frequently patient due to inability or reluctance to travel to the various research sites for sample collection at 48 hours and 3 months. Unfortunately the study was unable to provide subsidised transportation or community CRN support for the majority of participants that were located across a very wide geographical area.

Almost all participants were discharged or repatriated to their referring hospitals within 48 hours of their coronary angiogram, often precluding direct interaction with the research team beyond this point. Some participants with impaired mobility were assisted with travel arrangements to their nearest participating hospital for follow up, with telephone support provided for others when necessary. GP involvement had been considered during the study design process, but was not implemented due to the complexity of REC and NIHR approval for the numerous GP practices that would have been required to participate, in addition to anticipated difficulties with sample collection and processing. Of the 100 patients enrolled in the trial, 10 were excluded prior to the 48 hour time point as 0 hour eGFR was  $> 60\text{ml/min}$ , despite an initial screening eGFR of  $< 60\text{ml/min}$ . The labile nature of eGFR results, largely attributable to necessary provision of adequate oral hydration and withdrawal of diuretics pre-procedurally was unavoidable in the study. A total of 12 patients were lost to follow up at 48 hours and another 5 patients at 3 months, mainly due to participant non-attendance and sampling errors, and as such it is possible that CIN and longer-term renal sequelae were not detected in these excluded participants.

The lower than expected accrual rate resulting from the various logistical and clinical challenges ultimately negatively impacted on the projected recruitment target of 364 patients, whilst there was concern that patients at higher risk of CIN may not have been adequately represented in the study due to the inherent difficulties involved in recruiting patients with poor health or restricted mobility. Despite early recognition of these issues and significant efforts to address them, including learning from participant feedback in focus group meetings and two major amendments to the protocol expanding recruitment to multiple research centres, these intrinsic difficulties persisted until the close of the study.

In order to examine whether a lower risk cohort of study participants had inadvertently self-selected, the predicted CIN risk of all enrolled participants was calculated using the widely adopted CIN risk score proposed by Mehran et al, which was compared with observed CIN incidence in both the audit and clinical trial.

**Table 30: Predicted CIN incidence based on Mehran risk score**

Mehran Score & Risk of CIN	Control group, n=37		RIPC group, n=41 (assuming no RIPC effect)	
	n	Predicted CIN, n	n	Predicted CIN, n
<5 (5% risk)	11	0.55	9	0.45
6-10 (14% risk)	18	2.52	20	2.8
11-15 (26.1% risk)	6	1.56	11	2.87
>15 (53.1% risk)	2	1.06	1	0.53
<b>Predicted CIN Total</b>	37	<b>5.69 (15.4%)</b>	41	<b>6.65 (16.2%)</b>
<b>Observed CIN Total</b>	37	<b>2 (5.4%)</b>	41	<b>2 (4.8%)</b>

Interestingly the calculated CIN risk in both the control (15.4%) and RIPC (16.2%) groups closely matches that seen in the clinical audit (~15%), compared with the much lower observed rate of CIN (5.4% and 4.8%) in the clinical trial. This suggests that the Mehran score may not be applicable to the ERICCIN study population who received significantly more efficacious CIN prophylaxis than the population studied by Mehran et al and indeed the cohort included in the initial CIN audit at UCLH.

Data from the audit revealed that only 55% of at-risk patients received i.v. sodium bicarbonate pre-hydration and only 74% received iso-osmolar contrast agents, whereas 100% of participants in the study received both prophylactic interventions. It is also worth noting that a significant number of patients included in the audit would not have been eligible for the clinical trial, including those aged over 85 years, those undergoing PPCI for STEMI and those with previous contrast exposure within one month, all high risk factors for CIN deemed to be too complex to have included in the study.

### **7.3.3 OPTIMAL CIN PROPHYLAXIS**

Rigorous adherence to the 'best practice' CIN prevention protocol brought about a dramatic improvement in CIN incidence and patient outcomes, beyond that easily achievable in routine clinical practice or as seen in prior observational studies. This was achieved by strict adherence to nephrotoxic medication withdrawal peri-procedurally, pre and post hydration with i.v. bicarbonate 1.26% and use of low volumes of iso-osmolar CM with extensive use of biplane imaging techniques (section 1.3.7).

#### **7.3.4 CIN DEFINITION**

Serial SCr measurements collected over a broader 48 -120 hour timeframe may have included later CIN presentations which were not detectable at the 48 hour time point, although the intensive nature of blood sample collection required would not have been feasible to achieve in this predominantly outpatient study group. Equally measurement of urinary output, the most sensitive early marker of CIN requiring invasive urinary catheterisation, would not have been appropriate in our study population. Other validated markers of CIN such as Cystatin C, Urinary Kim-1 and L-FABP may have been of interest to study, although their correlation with adverse clinical outcomes following CIN is less well established.

#### **7.3.5 PARTICIPANT FEEDBACK GROUP**

Confounders may have existed during the feedback process as the group of patients who had devoted additional time and effort to attend were part of a cohort of patients who had already consented to be part of the trial. Although results from the feedback sessions were largely favourable and did not suggest a major revision of the trial design was required, it is impossible to know the opinions of those who did not wish to participate in the study at the outset. The attending participants were further sub-selected into a group who were self-motivated to attend the meeting and other non-attending participants' views remain unknown. As such any conclusions drawn from the feedback exercise should be interpreted with a degree of caution.



## **7.4 RE-AUDIT**

The start of the ERICCIN trial brought with it an educational campaign at participating research centres as the trial mandated 100% compliance with existing best-practice standards defined in the study protocol. Re-audit of clinical practice at UCLH, 12-months after the start of the trial, demonstrated improved compliance with CIN guidelines (60% compliance with pre-hydration, 91% compliance with Visipaque usage and mean contrast volume reduction from 160ml to 147ml in at-risk patients). Improved compliance was associated with a concomitant fall in the rate of CIN to between 6-14%, from a previous rate of 10-20%. As such it is possible to draw the conclusion that strict adherence with CIN guidelines is extremely effective at reducing the rates of CIN and that the trial functioned as an effective basis for an excellent quality improvement programme.

## **7.5 OTHER FINDINGS**

Remote ischaemic conditioning was found to be both safe and easy to apply, with no reported adverse events directly related to RIPC administration. The trial allowed robust assessment of the novel 'Deric' device, paving the way for use of the device in ongoing RIPC trials at UCLH. ERICCIN was a well-run, NIHR CRN approved multicentre clinical trial, which encouraged participant involvement throughout the study, receiving positive feedback from participants and members of the research teams.

## 7.6 POTENTIAL CAUSES FOR NON-SUPERIORITY OF RIPC

The neutral outcome of the ERICCIN study suggests that RIPC may not be an effective prophylactic treatment against CIN, although the study was not adequately powered to draw firm conclusions. This is in contrast to a number of previous pilot studies suggesting the efficacy of RIPC against CIN, the prototypical example being that performed by Er et al. However important differences exist between ERICCIN and this previous study, which enrolled a population at significantly higher risk of CIN with a median Mehran Score of 13 (Q1-3, 10 - 17) as compared with a Mehran score of 8 (Q1-3, 5 - 11) in the ERICCIN trial. Er et al also utilised a different CIN prevention strategy (PO NAC and i.v. 0.9% NaCl 1ml/kg hr for 12 hours pre and post CA with non iso-osmolar contrast agent use) and had higher rates of acute heart failure necessitating i.v. diuretic administration. This suggests that RIPC may be an effective intervention against CIN in higher risk patient groups and to a lesser degree in lower risk patients receiving effective standard prophylaxis.

The recently published RIPCIN study<sup>432</sup> (n=76), which followed a similar protocol to ERICCIN, also demonstrated no significant reduction in CIN at 48 hours after RIPC (2 sham vs 2 RIPC). However subgroup analysis of patients with Mehran risk scores  $\geq 11$ , showed a significant reduction in SCr from baseline to 48 - 72 hours in the RIPC group ( $\Delta$  SCr  $-3.3 \pm 9.8 \mu\text{mol/L}$ ) vs control group ( $\Delta$  SCr  $+17.8 \pm 20.1 \mu\text{mol/L}$ ). Subgroup analysis of ERICCIN study patients with Mehran scores  $>11$  unfortunately did not reflect this finding ( $\Delta$  SCr  $+5.0$  vs  $+4.5 \mu\text{mol/L}$ ), however the small number of patients in both groups (RIPCIN n=11, ERICCIN n=20) limits direct comparison and interpretation. It is reasonable to hypothesise that the highly effective CIN protocol used in ERICCIN, in addition to various confounders, may have led to under-powering of the study, suppression of a significant RIPC effect and a neutral outcome.

### 7.6.1 CLINICAL DIFFERENCES BETWEEN STUDY GROUPS

The minimised randomisation process robustly ensured that no significant differences existed between the control and RIPC groups with respect to the most important CIN risk factors, namely age >75 (35.1% vs 39%,  $p=0.54$ ), diabetes mellitus (48.6% vs 53.7%,  $p=0.84$ ), CCF (10.8% vs 12.1%,  $p=0.85$ ) and low haematocrit (32.4% vs 31.4%,  $p=0.29$ ). Contrast media volumes were equivalent in both groups (120ml vs 110ml,  $p=0.87$ ) as were total i.v. bicarbonate hydration volumes (750 ml vs 714ml,  $p=0.26$ ) and time to procedure after sham or RIPC which was performed largely within the 2 hour therapeutic window (80%). Apart from three variables which had not been expected nor accounted for in the study design, there were no significant differences in other demographic or clinical characteristics between the two groups:.

By chance the control group had a significantly higher proportion of patients with CCS I-IV anginal symptoms (48.6% vs 24.3%,  $p=0.03$ ) and perhaps unsurprisingly increased use of nitrate medications (51.4% vs 22%,  $p=0.007$ ) and P2Y<sub>12</sub> receptor antagonists (81.1% vs 61%  $p=0.05$ ). It is reasonable to postulate that, by chance, a beneficial set of confounders existed in the control group resulting in reduced risk of CIN when compared with the RIPC group, as discussed below.

### 7.6.1.1 Myocardial Ischaemia

The potential for myocardial ischaemia to subsequently induce RIPC on the kidney has not been extensively investigated. Deferios et al<sup>191</sup> demonstrated that in 225 patients undergoing PCI following NSTEMI, brief cycles of myocardial ischaemia induced by intermittent balloon inflation within the acutely stented coronary artery (RIPostC) significantly reduced the incidence of CIN (29.5% vs 12.4%,  $p=0.002$ ) and suggested a trend towards reduced mortality and rehospitalisation at 30 days. In light of this fascinating result, the higher burden of myocardial ischaemia present in the control group might theoretically have exerted a RIPC effect on the kidney, potentially via the ‘second window of protection’ phase, thus conferring reno-protection against CIN.

### 7.6.1.2 Nitrate use

The increased use of nitrate medications in the control group may have led to unintentional pharmacological conditioning against CIN (section 1.5.3.4). This phenomenon was proposed by Peguero et al<sup>438</sup> in 2015, following a retrospective analysis of 199 patients undergoing PCI following NSTEMI, where peri-procedural nitrate use was independently associated significantly lower rates of CIN (OR 0.334, CI 0.16-0.7,  $p= 0.03$ ), particularly when administered intravenously. The ERIC-GTN trial<sup>439</sup> is currently investigating the role of intravenous glyceryl trinitrate (GTN) as a cardio-protective agent during cardiac surgery and may determine whether GTN interferes with RIPC cardio-protection. Further investigation in large randomised studies is required to determine whether this simple and cost effective therapy can be successfully employed as prophylaxis against CIN.

### 7.6.1.3 P2Y(12) receptor antagonist use

The effect of P2Y(12) receptor antagonists (Clopidogrel, Prasugrel and Ticagrelor) on CIN is as yet unknown. Clopidogrel inhibits expression of platelet activation markers and platelet-leukocyte aggregation and has been shown to exert independent cardio-protective effects against myocardial IRI to the same magnitude as conferred by IPostC following PPCI in STEMI<sup>440</sup>. Both Ticagrelor and Cangrelor (an intravenous P2Y(12) receptor antagonist), have been shown to induce cardio-protection in animal models and do not confer additional benefit when used in combination with IPC<sup>441</sup>. This suggests P2Y(12) pharmacological conditioning and IPC may share a common pathway, recently suggested by Cohen et al<sup>442</sup> to be mediated by sphingosine kinase, rather than via platelet inhibition per se. Further studies are required to determine if the pharmacological conditioning effect of P2Y(12) receptor antagonists extends to the kidney and is effective against CIN.

### 7.6.2 RIPC RESISTANCE IN DIABETES MELLITUS

A large proportion of diabetic patients were enrolled the study (48.6% control group, 53.7% RIPC group) which may have led to significant abrogation of any RIPC effect. A number of studies have demonstrated that the pro-survival pathways critical to IC efficacy are impaired in diabetes mellitus<sup>443</sup>. Although most studies have investigated resistance to IC cardio-protection, it is reasonable to hypothesise that this inhibitory effect extends to reno-protection. In order to summate any resistance to RIPC in diabetic patients, the protocol deliberately utilised 4 cycles of RIPC, as opposed to 3 cycles as commonly used in previous studies, although it is unknown if increasing the number of RIPC cycles is sufficient to overcome this phenomenon. In practical terms, a higher number of RIPC cycles beyond that used in the study may lead to both logistical and tolerability limitations for the treatment.

## 7.7 CIN AND MICROALBUMINURIA

Few previous studies have directly examined the relationship between CIN and an acute changes in UACR, with most focusing on elevated UACR as a pre-procedural risk factor<sup>444</sup>, or associated more generally with AKI. Tzakias et al<sup>445</sup> compared the prognostic ability of three novel biomarkers of renal injury (NGAL, Cystatin C, IL-18) with UACR to detect AKI in patients hospitalised with MI. Acute elevation in UACR ( $> 66.7 \mu\text{g}/\text{mg}$  at 48 hours) was found to be an independent marker of AKI (Sensitivity 68%, Specificity 76%) with superior discriminating ability over urine NGAL, urine Cystatin-C and serum Cystatin-C across a wide variety of clinical sub-groups. Although not powered to determine an association of UACR with clinical outcomes, development of AKI was associated with prolonged hospitalisation, increased morbidity during hospital admission, persistent worsening of renal function and increased mortality at follow-up.

Animal models have shown that UACR increases as early as 4 hours after intrinsic renal injury (ischemia–reperfusion, nephrotoxicity and rhabdomyolysis) although not in pre-renal (endotoxin) or post-renal (obstructive uropathy) injury<sup>446</sup>. These findings suggest that acute elevation in UACR is specific to intrinsic causes of AKI such as CIN and precedes changes in SCr. As such this simple bedside test appears to be a practical and cost effective tool to safely rule-out or predict the onset of CIN.

ERICCIN is the first clinical trial to examine the effect of RIPC on CIN using UACR as a biomarker of renal injury, however it was not designed to assess whether UACR could predict CIN, as defined by elevation in SCr. Although no significant reduction in UACR was demonstrated following RIPC despite a general trend towards benefit, subgroup analysis in patients with CCF showed significant benefit and a near significant benefit was seen in patients with anaemia and a Mehran score  $>6$ .

This interesting finding may be worthy of future study, not least due to the fact that UACR is an established biomarker for predicting adverse cardiovascular events and mortality<sup>447</sup>. This subgroup of patients with established higher cardiovascular risk, inherent to conditions such as CCF, might potentially have more to gain from therapeutic interventions designed to minimise elevation in UACR. Large adequately powered RCT's are required to establish whether RIPC is effective at preventing CIN and acute elevation of UACR in higher risk groups and whether this correlates with improved short and long-term cardio-renal outcomes.

## CHAPTER 8: CONCLUSION & FUTURE DIRECTIONS

At the outset of the study, CIN was established to be a common and serious complication occurring in high risk patients undergoing CA or PCI at UCLH, in keeping with the wealth of supporting evidence from previous studies within this area. Adherence to the local and national standard of care for CIN prophylaxis was found to be suboptimal, prompting a local educational programme and audit cycle whilst inspiring development of the randomised controlled trial ERICCIN, designed to examine whether RIPC, a safe, easily applied and non-invasive therapy, in addition to standard care was capable of further minimising the incidence of CIN, a prototypical example of renal ischaemia reperfusion injury.

Out of a target of 362 patients at risk of CIN following CA or PCI, 100 participants were recruited across three hospital research sites in London and South East of England and randomly allocated to RIPC (four 5 minute cycles of upper limb ischaemia-reperfusion) plus standard therapy vs standard therapy alone, with renal injury biomarkers (SCr and UACR) and cardio-renal endpoints assessed over 48 hour and 3 month time points. Due to an unexpectedly low incidence of CIN in the control group, the study was significantly underpowered and was closed prematurely by the study DMC, with no significant difference in CIN rate, novel biomarkers or cardio-renal endpoints between groups occurred by close of study.

Whilst the ERICCIN trial was unable to demonstrate the efficacy of RIPC against CIN within the study cohort, it was found that optimal management of high risk patients consistent with 100% compliance with evidence based local and national guidelines, all but eradicated the excess kidney injury associated with contrast administration in the study cohort.



Implementation of the comprehensive education and audit cycle in parallel with the study brought about significant improvements in standards of care and improved patient outcomes. This of itself was an unexpected but welcome finding, which fully justifies full implementation of current clinical guidance in this field of practice.

Despite the neutral outcome of the study, several hypothesis-forming findings were uncovered, including whether RIPC might be more efficacious in patients at very high-risk of CIN and the potential for minimisation of UACR elevation. Despite optimal CIN prophylaxis in very high-risk groups, cardio-renal outcomes remain predictably poor and often necessitate costly and harmful interventions. RIPC may offer a simple and cost neutral therapy for carefully selected very high risk patients, where even a modest beneficial effect has the potential to dramatically improve patient outcomes and reduce healthcare costs. The concept of a threshold for RIPC reno-protection is raised by the high number of participants with diabetes mellitus within the study group who may not have been responsive to the RIPC protocol, perhaps requiring greater frequency or longer duration of limb ischaemia reperfusion cycling or even combination with alternative forms of conditioning. It is possible that inadvertent myocardial RIPC or pharmacological conditioning of the control group by nitrate medications and P2Y<sub>12</sub> inhibitors may have conferred a similar protective effect against CIN as that of RIPC in our study group.

These phenomenon are worthy of further study in their own right, with the latter currently the focus of ongoing basic and clinical research at UCLH. Development and validation of the novel 'DERIC' device is also expected to significantly enhance, homogenise and simplify the delivery of RIPC in these future clinical trials. The results of our study do not rule out RIPC as a prophylactic adjunct against CIN, but suggests that it may only be of benefit in certain populations and clinical scenarios, the recommended primary foci for future studies.

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# APPENDIX

## 1. DERIC safety certificate

### Medical Engineering Solutions Ltd

99 Issigonis House  
London, W3 7UN

Tel: +44(0)203 290 7920

Email: med.eng.solutions.ltd@gmail.com

Company Registration No.: 07999692

### Electrical Safety Report:

ES14010

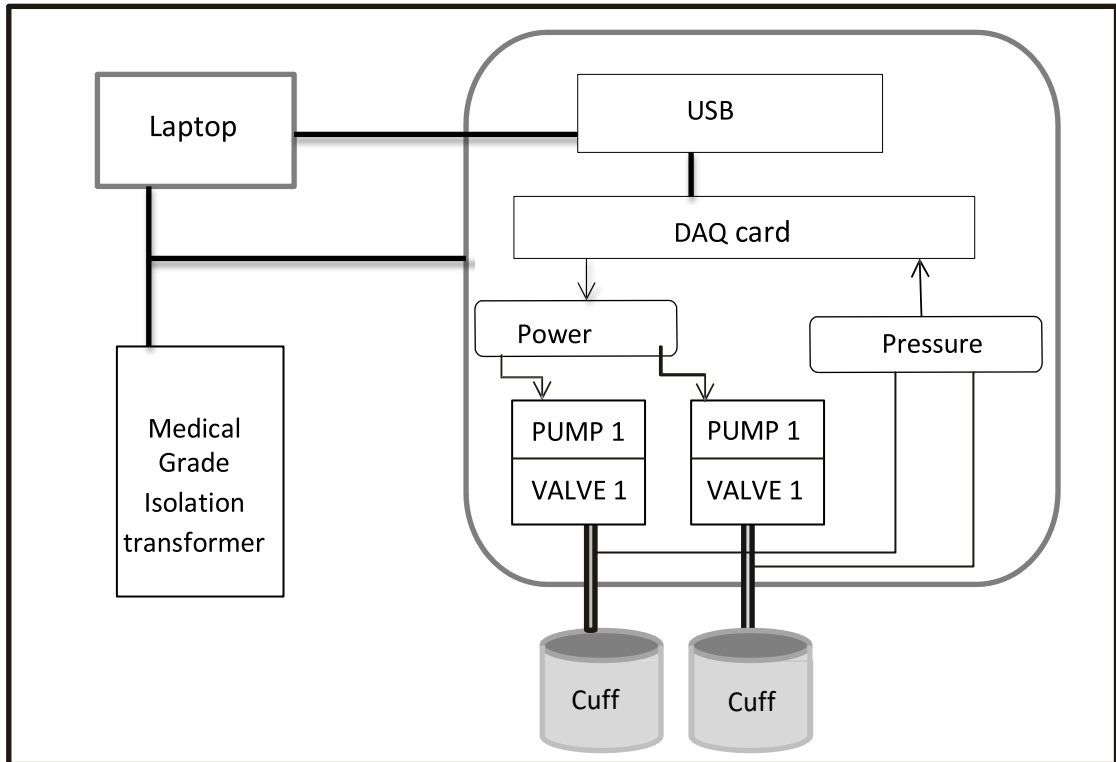
<b>Account Name:</b>	UCL - DERIC
<b>Address :</b>	Room 3.23, Dept. Medical Physics and Bioengineering, Malet Place Engineering Building, University College London, Gower Street, LONDON, WC1E 6BT
<b>Contact name:</b>	Dr Nick Everdell/Roger Rear

### Medical Equipment information:

<b>Manufacturer:</b>	UCL	<b>Model:</b>	DERIC System	<b>Serial Number:</b>	N/A
<b>Description:</b>	A laptop with control software, controlling DERIC system for controlled operation of inflation pumps.				
<b>Class:</b>	Class I BF				



## 1. Block Diagram



### Electrical Safety Conditions:

#### Mains Voltage:

L1-earth	239V
L2-earth	0.5V
L1-L2	240V

#### Protective Earth Resistance (<0.20 Ω):

0.11 Ω	<b>Pass</b>
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#### Insulation Resistance (>20.00 MΩ)

>99.99 MΩ	<b>Pass</b>
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#### Earth Leakage Current:

Normal:	0.041mA	<b>Pass</b>
nP:	0.060mA	<b>Pass</b>
rP:	0.100mA	<b>Pass</b>
rEu:	0.098mA	<b>Pass</b>

### Enclosure Leakage Current

Normal:	0.040mA	Pass
nE:	0.040mA	Pass
nP:	0.040mA	Pass
rP:	0.040mA	Pass
rEu:	0.040mA	Pass
rE:	0.086mA	Pass

### Patient Leakage Current (AC)

Normal:	0.004mA	Pass
nE:	0.004mA	Pass
nP:	0.004mA	Pass
rP:	0.004mA	Pass
rEu:	0.004mA	Pass
rE:	0.004mA	Pass

### Patient Leakage Current (DC)

Normal:	0.004mA	Pass
nE:	0.004mA	Pass
nP:	0.004mA	Pass
rP:	0.004mA	Pass
rEu:	0.004mA	Pass
rE:	0.004mA	Pass

### Patient Auxiliary Current (AC)

Normal:	0.004mA	Pass
nE:	0.004mA	Pass
nP:	0.004mA	Pass
rP:	0.004mA	Pass
rEu:	0.004mA	Pass
rE:	0.004mA	Pass

### Patient Auxiliary Current (DC)

Normal:	0.004mA	Pass
nE:	0.004mA	Pass
nP:	0.004mA	Pass
rP:	0.004mA	Pass
rEu:	0.004mA	Pass
rE:	0.004mA	Pass

**Patient F-type Current**

Normal:	0.025mA	<b>Pass</b>
nE:	0.025mA	<b>Pass</b>
nP:	0.025mA	<b>Pass</b>
rP:	0.025mA	<b>Pass</b>

**Load**

<0.06 KVA (239V)	<b>Pass</b>
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**Polarity**

Open	<b>Pass</b>
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**Comments:**

The above readings all fall within safety limits. Appropriate electrical safety should be repeated if equipment transported to different location.

<b>Print</b>	<b>Sifis Nikiforos</b>	<b>Signature:</b>	<b>Sifisn</b>	<b>Date:</b>	<b>21/11/2013</b>
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