FINAL REVISION OF THESIS

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Effects of remote ischaemic preconditioning on peri-operative myocardial injury and clinical outcomes in patients undergoing elective cardiac bypass surgery

1) I acknowledge that in tables indicating patients' baseline characteristics, indication of p value is not appropriate.

2) I acknowledge that AUC of hsTnT should not be expressed in µg/L.
   The correct unit for AUC of hsTnT is: µg/L · hr (i.e. concentration multiplied by time) and this applies to each time it is reported in the thesis.

3) Pag. 142: Table 3.11. Summary of major secondary endpoints in patients receiving cardioplegia.
   The following figures are correct:
   - in Control group: death=5, myocardial infarction=1, stroke=0, revascularization=0
   - in RIPC group: death=0, myocardial infarction=0, stroke=1, revascularization=0

4) Pag. 246: Fig. 6.7. Mean high-sensitivity Troponin-T levels at 0, 6, 12, 24, 48 and 72 hours in diabetic patients undergoing CABG surgery (mean±SEM). Figure is incorrect as it represents a similar reproduction of Fig. 6.8.
   The correct figures are:
   a) Control patients.
      hsTnT0: 0.024 (0.006) µg/L; hsTnT6: 0.709 (0.080) µg/L; hsTnT12: 0.653 (0.067) µg/L, hsTnT24: 0.449 (0.073) µg/L; hsTnT48: 0.389 (0.078) µg/L; hsTnT72: 0.368 (0.061) µg/L; AUC: 31.73 (4.52) µg/L · hr

b) RIPC patients.
hsTnT0: 0.016 (0.006) µg/L; hsTnT6: 0.544 (0.069) µg/L; hsTnT12: 0.459 (0.048) µg/L; hsTnT24: 0.302 (0.033) µg/L; hsTnT48: 0.217 (0.024) µg/L; hsTnT72: 0.158 (0.021) µg/L; AUC: 19.63 (2.23) µg/L · hr

Signed

Luciano Candilio

Luciano Candilio 19/04/2015

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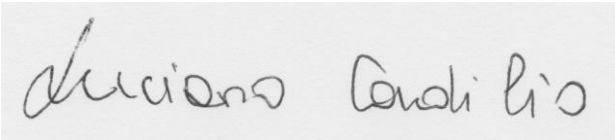
Declaration

I, Luciano Candilio, confirm that the work presented in this thesis is my own. Information that has been derived from other sources has been clearly indicated and referenced within my thesis. The thesis presented is the one on which I expect to be examined.

Signed



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Date 27/11/14

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Abstract

Ischaemic heart disease (IHD) is a major cause of morbidity and mortality in the world. Coronary artery bypass graft (CABG) surgery is the revascularisation strategy of choice in a significant number of patients, particularly those with diabetes mellitus and complex coronary disease. During cardiac surgery, the myocardium is subjected to peri-operative myocardial injury (PMI), which has been associated with worse short and long-term clinical outcomes. Higher-risks patients are currently being operated on with subsequent higher risk of PMI and worse prognosis: therefore new strategies are required to potentiate the innate mechanisms of cardioprotection. In this regard, remote ischaemic preconditioning (RIPC) is a promising non-invasive intervention able to reduce PMI in these patients: however, not all the studies have shown significant cardioprotection with RIPC for a number of factors, amongst which the intensity of the preconditioning stimulus may play a significant role.

We therefore investigated whether an enhanced RIPC stimulus, given with transient simultaneous multi-limb ischaemia/reperfusion, was able to reduce PMI and improve short-term clinical outcomes in patients undergoing elective cardiac surgery: we demonstrated that our preconditioning stimulus can significantly reduce PMI, length of intensive care unit (ICU) stay and incidence of atrial fibrillation (AF) in these patients. In addition, further retrospective analyses showed improved myocardial protection in preconditioned diabetic patients undergoing CABG surgery and in control CABG subjects receiving combined antegrade and retrograde cardioplegia compared to control CABG patients having antegrade cardioplegia or intermittent cross-clamp-fibrillation.

We also conducted a multi-centre, double-blinded randomised control clinical trial, in which we investigated the effects of RIPC on clinical outcomes at 1 year in high-risk

patients undergoing elective CABG surgery with or without valve surgery (the ERICCA trial). The results of this study are due to be presented in March 2015 and have the potential to significantly impact on clinical practice in cardiac surgery.

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List of Abbreviations

6MWT - 6-minute walk test

A1R - adenosine receptor

AAA - abdominal aortic aneurysm

ACE-I - Angiotensin Converting Enzyme-Inhibitor

ACS - acute coronary syndrome

ADP - adenine diphopshate

AF - atrial fibrillation

AIDS - acquired immunodeficiency syndrome

AKI - acute kidney injury

ALI - acute lung injury

ALT - alanine transaminase

AMI - acute myocardial infarction

ANP - atrial natriuretic peptide

ANT - adenine nucleotide translocase

AR - aortic regurgitation

ARB - angiotensin receptor blocker

ATP - adenine triphopshate

AUC - area under the curve

AV - aortic valve

AVR - aortic valve replacement

BCS - British Cardiovascular Society

BK - bradykinin

BNP - brain natriuretic peptide

BP - blood pressure

BUN - blood urea nitrogen

CABG - coronary artery bypass graft

CAD - coronary artery disease

CAS - carotid artery stenting

CB - endocannabinoid

CCB - calcium-channel blocker

CCS - Canadian Cardiovascular Society

CEA - carotid endarterectomy

CGRP - calcitonin gene-related peptide

CI - confidence interval

CIN - contrast-induced nephropathy

CK- creatine kinase

CKD - chronic kidney disease

CMR - cardiac magnetic resonance

COPD - chronic obstructive pulmonary disease

CPB - cardiopulmonary bypass

CRF - case report form

CRP - C-reactive protein

CV - cardiovascular

CVA - cerebrovascular accident

CVA - cerebro-vascular accidents

CVD - cardiovascular diseases

Cx - circumflex

DMC - Data Management Committee

DM - diabetes mellitus

DVR - double valve replacement

DVT - deep vein thrombosis

ECG - electrocardiogram

eGFR - estimated glomerular filtration rate

EVAR - endoscopic abdominal aortic aneurysm

EVC - Endpoint validation committee

FDG - $^{18}F_2$-fluorodeoxyglucose

FEV - Forced Expiratory Volume

FiO2 - Fraction of inspired Oxygen

GCP - Good Clinical Practice

GP - general practitioner

GPCR - Gi protein-coupled receptor

GTN - glyceryl trinitrate

HRQOL - Health-Related Quality of Life

hsTnT - high-sensitivity troponin T

IABP - intra-aortic balloon pump

ICCF - intermittent cross-clamp fibrillation

ICH- - International Conference of Harmonisation

ICU - intensive care unit

IGF - insulin-like-growth factor

IHD - ischaemic heart disease

IL - interleukin

IMA - internal mammary

INR - International Normalized Ratio

IPC - ischaemic preconditioning

IPost - ischaemic post-conditioning

IQR - interquartile range

IR - ischaemia reperfusion

IRI - ischaemia reperfusion injury

IV - intravenous

K_{ATP} - potassium sensitive adenine triphosphate

LAD - left anterior descending

LBBB - left bundle branch block

LDH - lactate dehydrogenase

LIMA - left internal mammary

LIPC - limb ischaemic preconditioning

LMS - left main stem

L-NAME - L-nitro-methyl-arginine ester

LSHTM - London School of Hygiene and Tropical Medicine

LV - left ventricle

LVEF - left ventricular ejection fraction

MACCE - major adverse cardiac and cerebrovascular events

MI - myocardial infarction

MITO-K_{ATP} - mitochondrial potassium sensitive adenine triphosphate

MMP - matrix metalloproteinase

MPO - myeloperoxidase

mPTP - mitochondrial permeability transition pore

MV - mitral valve

MVD - multi-vessel disease

MVR - mitral valve replacement

NADPH - Nicotinamide adenine dinucleotide phosphate

NGAL - neutrophil gelatinase-associated lipocalin

NO - nitric oxide

NSTEMI - Non-ST Segment elevation myocardial infarction

NYHA - New York Heart Association

OMT - optimal medical therapy

PAD - peripheral arterial disease

PaO2 - oxygen tension

PCI - percutaneous intervention

PE - pulmonary embolism

PICO - Population, Intervention, Comparator, Outcome)

PKC - protein kinase C

PKG - protein kinase G

PMG - Program Management Group

PMI - peri-operative myocardial injury

PPCI - Primary Percutaneous Intervention

RBP - retinol binding protein

RCT - randomised control trial

RIPC - remote ischaemic preconditioning

RIPerC - remote ischaemic per-conditioning

RIPostC - remote ischaemic post-conditioning

RISK - Reperfusion Injury Salvage Kinase

ROS - reactive oxygen species

RR - relative ratio

RV - right ventricular

RVEF - right ventricular ejection fraction

SAE - serious adverse event

SAFE - survivor activator Factor Enhancement

SAX - short-axis

SBP - systolic blood pressure

SD - standard deviation

SEM - standard error of the mean

SMD - standardised mean difference

SNOSE - Sequentially Numbered Opaque Sealed Envelopes

SOD - superoxide dismutases

SPT - sulphophenyltheophylline

STEMI - ST-Segment elevation myocardial infarction

SYNTAX - Synergy between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery

TAAA - thoraco-abdominal aortic aneurysm

TGF - transforming growth factor

TIA - Transient Ischaemic Attack

TnC - troponin C

TNF - tumor necrosis factor

TnI - troponin I

TnT - troponin T

TSC - Trial Steering Committee

TSPO - translocator protein

TTE - transthoracic echocardiogram

TV - tricuspid valve

UA - unstable angina
UCL - University College London
UCLH - University College London Hospital
ULN - upper limit of normal
URL - upper range limit
VSD - ventricular septal defect
WHO - World Health Organization

CHAPTER 1

1. Introduction

1.1. Epidemiology of Coronary Heart Disease.

IHD remains a major cause of morbidity and mortality in the world despite significant advances in diagnostic and therapeutic measures over the last decades: in 2012 an estimated 56 million people died worldwide (http://www.who.int/mediacentre) and cardiovascular diseases (CVD), cancer, diabetes mellitus (DM) and chronic lung diseases represented the main causes **(Table 1.1).** CVD accounted for the death of 17.5 million people in 2012 (3 in every 10 deaths) (2, 3), of whom 7.4 million people died of IHD and 6.7 million from stroke, which correspond to 13.2% and 11.9% respectively of the total number of deaths in the world (3). IHD is the leading cause of death in high-income countries and lower-middle countries (4), and its incidence continues to rise in upper-middle and low-middle countries (3), which follows the dramatic increase in cardiovascular risk factors in these regions (5-7). Crucially, following an ST-Segment Elevation MI (STEMI) mortality rate remains as high as 2.5-10%, with slightly better outcomes (approximately 2-4%) in Non-ST Segment Elevation MI (NSTEMI)(8, 9), and with an overall estimated rate of 10% of in-hospital heart failure or shock, 6-7% of re-infarction at 1 year, 1.8% of in-hospital major bleeding, and 1.8-2% stroke at 1 year (10). Crucially, IHD also represents a significant economic burden for health care systems: in 1999, IHD accounted for an estimated total NHS cost of £7055 billion (11), due to direct and informal healthcare costs and productivity loss (11).

Table 1.1. The ten leading causes of death in the world in 2012 compared to 2000

Disease	N (million) in 2012 (2010)	%
Ischaemic heart disease	7.4 (6)	13.2
Stroke	6.7 (5.7)	11.9
COPD	3.1 (3.1)	5.6
Lower respiratory tract infections	3.1 (3.5)	5.5
Trachea, bronchus, lung cancer	1.6 (1.2)	2.9
HIV/AIDS	1.5 (1.7)	2.7
Diarrhoeal disease	1.5 (2.2)	2.7
Diabetes mellitus	1.5 (1)	2.7
Road injury	1.3 (1)	2.2
Hypertensive heart disease	1.1 (0.8)	2

COPD=chronic obstructive pulmonary disease; HIV=human immune-deficiency virus; AIDS=acquired immunodeficiency syndrome

Appropriate treatment strategies are therefore required in order to reduce morbidity and mortality secondary to IHD and revascularization with either percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG) surgery provide the best therapeutic approaches. The choice of treatment strategy varies according to clinical presentation, coronary anatomy, presence of co-morbidities, concomitant therapy, prognostic advantages, and patient's preference (12). In patients with stable angina, CABG is the revascularisation treatment of choice when (13-15):

a) their symptoms are not satisfactorily controlled with optimal medical therapy (OMT) and revascularisation is considered appropriate albeit not achievable with PCI;

b) both procedures would be suitable, however patients present multi-vessel disease, their symptoms are not satisfactorily controlled with OMT, have DM or are over 65 years or have anatomically complex three-vessel disease, with or without involvement of the LMS: in theses case, CABG has been demonstrated to offer a survival advantage over PCI (13-15).

1.2. Historical background and techniques of myocardial preservation during cardiac surgery

Since the first application of cardiac surgery in 1953, when John Gibbon performed the first surgical closure of atrial septal defect, an impressive advancement of surgical techniques has occurred over the last few decades, with crucially the introduction of methods aiming at preserving myocardial function in the peri-operative period (16). The concept of myocardial preservation during cardiac surgery became soon of critical importance in the success of the operation and patient's survival. Against the significant risks of air embolism and blood loss of the operation with "a beating heart", the technique of cross-clamp of the aorta was introduced in order to ensure a dry operative field. This was also able to induce transient global ischaemia, during which time a cardiopulmonary bypass (CPB) machine would provide organ oxygenation and perfusion. However global ischaemia itself, albeit transient, was associated with significant PMI, which then led to poor patient outcomes. Therefore the concepts of PMI and myocardial preservation or cardioprotection became closely interrelated and have been a major area of interest in research over the last few decades.

Hypothermia with or without cardio-circulatory arrest was the first method of myocardial preservation to be introduced (17, 18), but was soon replaced by the use of "cardioplegic" solutions, able to induce cardiac arrest due to a high potassium concentration. However due to the significant myocardial damage observed with this technique, it soon became "obsolete" in the 1960s. During this time, hypothermic arrest with continuous coronary perfusion was further improved with the introduction of ventricular fibrillation, which uses an alternating current in order to induce ventricular standstill. Importantly, the latter was soon found to cause subendocardial ischaemia

through an increase in left ventricular (LV) end-diastolic pressure during perfusion and was then used in combination with intermittent cross-clamp of the aorta, which instead causes transient global ischaemia of the myocardium (19): the resulting technique was therefore termed intermittent cross-clamp fibrillation (ICCF) and partially obviated the relative myocardial damage induced by both techniques (20).

Interestingly, in the 1970s there was a renewed interest in the cardioplegic strategy, in view of the significant myocardial ischaemia induced with the first technique of ICCF and new forms of cardioplegia were therefore developed, such as hypothermic, normothermic, crystalloid, substrate-enhanced and blood cardioplegia (21-24). In particular the St. Thomas cardioplegia solution-1, which introduced the era of crystalloid cardioplegia, whilst allowing good visualisation of the operating field, it also caused a slow recovery of myocardial function and aerobic myocardial metabolism, hence facilitating lactate production and myocardial injury (25). Crystalloid solutions have high potassium concentration, buffers such as amino acids and bicarbonate, and various substances increasing oncotic activities, including mannitol, lidocaine, procaine, plus low (Bretschneider's solution) or high (St. Thomas cardioplegia solution 2) calcium content (with low sodium in the former and high magnesium in the latter) (26). Conversely, blood cardioplegic solutions consist of native blood and a crystalloid solution in a ratio of 4:1, and have potassium in high concentrations and calcium in low concentrations (respectively to allow cardioplegic arrest and prevent cardiomyocyte apoptosis and necrosis), buffers, amino acids (to facilitate aerobic metabolism and high oncotic pressure), and glucose content (to prevent myocardial oedema) (27). This leads to important advantages of blood over crystalloid cardioplegia on cardiomyocyte protection, such as the versatility in maintaining an oncotic balance, the buffering and anti-oxidant properties and oxygen

delivery (27, 28), thereby accelerating the recovery of myocardial aerobic metabolism and reducing reperfusion damage (29). A survey conducted in the UK and Ireland in 2004 estimated that approximately 16% of surgeons use ICCF and 84% cardioplegia, of whom 83.5% use blood and 16.5% crystalloid cardioplegia (30). However, due to its shorter ischaemic times (31), ICCF remains the preferred technique of myocardial preservation in patients with pre-operative conduction abnormalities (it presents low incidence of conduction complications), or with a permanent pacemaker (which needs to be disconnected in the context of cardioplegia) or with cold agglutinins, as in this case a normothermic field and therefore shorter ischaemic times are required (32).

In summary, despite very significant improvements of the above-mentioned techniques of myocardial preservation, the level of PMI sustained during cardiac surgery is still considerable, partly because of the relative inadequacy of these methods and partly because of the change in risk profile of patients being operated on, with a subsequent relevant impact on short and long-term clinical outcomes in these subjects.

1.2.1. Short and long-term clinical outcomes in patients undergoing cardiac surgery

Over the last few decades the profile of patients undergoing cardiac surgery has significantly changed due multiple factors including the ageing population, the presence of co-morbidities such as diabetes and hypertension, more complex CAD being operated on, and concomitant valve surgery, all of which increase peri-operative risk by exposing the patient to peri-operative complications (33, 34). These include:

1. Myocardial dysfunction, which can be a consequence of:
    a. Acute spasm or occlusion of a coronary graft, prosthetic or

paraprosthetic valve regurgitation, cardiac tamponade, pneumothorax, haemothorax.

   b. Inadequate preload, excessive afterload, impaired ventricular function due to ischaemic event and myocardial reperfusion injury in the intra-operative period (this will be discussed later).
   c. Tachy-bradyarrhythmias, including AF, ventricular arrhythmias, high degree atrio-ventricular block.
   d. Peri-operative myocardial infarction (discussed later)

2. Vasodilatory shock, due to systemic inflammatory response to ischaemia and reperfusion and to the exogenous substances included in the cardioplegic solution.
3. Haematological dysfunction, causing thrombosis or bleeding, due to platelet and coagulation cascade abnormalities, residual heparin effect, incomplete surgical haemostasis.
4. Pulmonary dysfunction, including pleural effusion, pneumonia, atelectasis, acute lung injury (ALI), diaphragmatic incompetence, endotracheal intubation complications.
5. Neurological dysfunction, such as cerebro-vascular accidents (CVA), coma, memory deficit, intellectual decline and seizures.
6. Renal dysfunction (this will be discussed later).
7. Mortality: currently peri-operative mortality rate in patients undergoing CABG surgery is approximately 1% for low-risk patients and 2-5% for the remaining patients (35-38), although there is considerable variation in the different centers (39). A series of factors have been implicated as potential contributory to mortality following cardiac surgery and include:

a) Previous cardiac centers experience: studies have proved that mortality rates are lower in those centers with high volume operations per year, particularly for patients at moderate and high risk (37, 38, 40-43).
b) The surgeon's experience, which is a mortality predictor independent of the hospital load but strictly correlated to the latter, with the lowest incidence of mortality with high-volume surgeons at high-volume centers, particularly for moderate to high-risk patients (38, 42).
c) LV function, which is a major risk factors for peri-operative mortality (44, 45) as poor LV function has been associated with a 6% increased risk of mortality (45).
d) Age: as previously described, an increasing number of elderly patients are being operated on (34), with a higher associated risk of in-hospital mortality and stroke (46)
e) Acute kidney injury (AKI): this will be discussed in more details in the discussion section of chapter 2.
f) Chronic kidney disease (CKD), which has been associated with both short and long-term post-operative mortality (1, 47-49) even in the presence of mild renal dysfunction (50), with an increasing risk as the renal function becomes more impaired (51).
g) The presence of new Q waves (52).
h) Arterial graft and coronary artery diameter: the use of arterial grafts such the IMA has been associated with a significantly reduced rate of in-hospital and long-term mortality (53, 54), with no significant difference if single or multiple arterial grafts are performed (55-57). In addition, patients with a relatively small diameter of the coronary arteries, particularly the left anterior

descending (LAD), have an increased peri-operative risk, likely secondary to technical difficulties, thrombosis and reduced graft patency (58-60)

i) Other rarer complications, which have an impact on peri-operative mortality in patients undergoing CABG surgery, are gastrointestinal (61), metabolic (62) and haematological (63-66).

Furthermore, risk factors of long-term mortality of patients undergoing cardiac surgery include:

1. Factors related to grafts used. The use of an IMA to LAD has been associated with better long-term clinical outcomes compared to the use of saphenous vein grafts (SVG) only (53, 54, 67-70).
2. PMI: this will be discussed later.
3. AKI and chronic kidney disease.
4. AF: this will be discussed later.
5. Pre-operative haemoglobin level (64).
6. Cardiovascular (CVS) risk factors including advanced age, hypercholesterolemia, diabetes, hypertension and smoking history, which have also been associated with increased mortality in patients undergoing CABG surgery (71-74).

Crucially, the identification of these risk factors has also provided the opportunity to develop algorithms able to predict mortality risk in patients undergoing cardiac surgery, which have proved to be a very useful guide to clinicians and patients on the advisability of the operation by weighing both risk and benefits (75): of these, the EuroSCORE (European System for Cardiac Operative Risk Evaluation) (1, 48, 76) (**Table 1.2**), is widely used in Europe and has recently also been adopted worldwide, due to its accurate predictive power of 1 and 12 months mortality.

Table 1.2. EuroSCORE mortality risk prediction algorithm in patients undergoing cardiac surgery (modified from *Nashef et al* (1))

Predictor	Definition	Points
Age	Per 5 years or part thereof over 60 years	1
Sex	Female	1
Chronic pulmonary disease	Long-term use of bronchodilators or steroids for lung disease	1
Extracardiac arteriopathy	Any one or more of the following: claudication, carotid occlusion or >50 percent stenosis, previous or planned intervention on the abdominal aorta, limb arteries or carotids	2
Neurological dysfunction	Disease severely affecting ambulation or day-to-day functioning	2
Previous cardiac surgery	Requiring opening of the pericardium	3
Serum creatinine	>200 mmol/L (2.3 mg/dL) preoperatively	2
Active endocarditis	Patient still under antibiotic treatment for endocarditis at the time of surgery	3
Critical preoperative state	Any one or more of the following: ventricular tachycardia or fibrillation or aborted sudden death, preoperative cardiac massage, preoperative ventilation before arrival in the anesthetic room, preoperative inotropic support, intra-aortic balloon counterpulsation, preoperative acute renal failure (anuria or oliguria <10 mL/hour)	3
Unstable angina	Rest angina requiring intravenous nitrates until arrival in the anesthetic room	2
LV dysfunction	Moderate or LVEF 30-50% Poor or LVEF<30%	1 3
Recent myocardial infarct	<90 days	2
Pulmonary hypertension	SPAP >60 mmHg	2
Emergency operation	Carried out on referral before the beginning of the next working day	2
Other than isolated CABG	Major cardiac procedure other than or in addition to CABG	2
Surgery on thoracic aorta	For disorder of ascending, arch or descending aorta	3
Post-infarct septal rupture		4

LV=left ventricular; LVEF=left ventricular ejection fraction; SPAP=systolic pulmonary artery pressure; CABG=coronary artery bypass graft.

It and can be used as both an additive or a logistic risk model: the former is a very useful “bedside” system, which enables the operator to classify patients undergoing cardiac surgery into, low, intermediate and high-risk **(Tables 1.3).** The latter uses logistic regression to calculate the risk of death. In our studies, we widely utilised the additive EuroSCORE due to its simplicity of use.

Table 1.3. Peri-operative Mortality risk in patients undergoing cardiac surgery based on additive EuroSCORE (modified from *Nashef et al(1)*)

EuroSCORE	Risk stratification	Peri-operative Mortality
0-2	Low risk	0.8% (0.56-1.1)
3-5	Intermediate risk	3% (2.62-3.51)
≥6	High risk	11.2% (10.25-12.16)

1.3. Peri-operative Myocardial Injury

Peri-operative myocardial injury (PMI) describes the damage sustained by the myocardium during an invasive procedure or operation. In the context of cardiac surgery it has been associated with worse short and long-term clinical outcomes (77-82) and a significant number of factors have been implicated as potential underlying mechanisms (83). They include:

1) *Direct myocardial damage*, due retraction and handling of the heart (83).
2) *Poor surgical technique*, which might results in the following complications (84):
    a. failure of the aorto-coronary bypass grafts due to inadequate distal anastomoses or poor harvesting technique of saphenous veins (85);
    b. prosthetic valve incompetence secondary to poor placement;
    c. incomplete revascularisation, due to failure to recognise significant CAD or to achieve full revascularisation in severely diseased coronary arteries (84);
    d. erroneous decision on repairing rather replacing a mitral valve (MV).
3) *Systemic inflammatory response* to extraneous substances in the CPB circuit coming into contact with patient's blood, direct surgical trauma, blood loss and hypothermia, which can induce the activation of complement cascade,

inflammatory mediators release including cytokines, chemokines, hormones, vasoactive substances, reactive oxygen species (ROS), enzymes, ultimately leading to severe inflammation, coagulation factors consumption, micro-embolisation and multi-organ failure (86, 87).

4) *LV over-distension,* which can particularly occur when CPB has been established and in the presence of significant aortic valve (AV) regurgitation, leading to retrograde blood flow through the incompetent AV and subsequently to LV distension and dysfunction (84).
5) *Coronary athero-embolization,* consisting in embolization of intracoronary thrombus or atherosclerotic particulate debris during coronary manipulation *(88).*
6) *Increased cardiac workload* in the intra-operative period (84).
7) *Ischaemic injury*, which can occur in the setting of both ICCF and cardioplegia as a consequence of intermittent cross-clamp of the aorta carried out during the attachment of the distal end of the graft anastomosis whereas the proximal end is constructed after declamping and therefore during the reperfusion phase.
    - In ICCF, despite the shorter cross-clamp times, the ischaemic insult is significant due to a combination of global ischaemia induced by cross-clamp and subendocardial ischaemia caused by ventricular fibrillation in the reperfusion phase.
    - In Cardioplegia, the more significant global ischaemia due to longer cross-clamp times is mitigated by the administration of cellular protective solutions initially and during each episode of aortic cross-clamp.

In summary, the magnitude of PMI in the context of ICCF and cardioplegia is essentially equivalent (31, 89-93) and the implications of this will be discussed in chapter 3.

8) *Genetic predisposition (84):* the presence of specific gene polymorphism has been associated with a pro-inflammatory state generating an “excessive” systemic inflammatory response to the above-mentioned factors, thereby leading to more significant PMI (94).
9) *Myocardial stunning*, a reversible contractile dysfunction secondary to reperfusion that follows global ischaemia induced by aortic cross-clamp (95).
10) *Myocardial ischaemia-reperfusion injury* (IRI): this describes the myocardial damage induced by the restoration of blood flow following a period of prolonged ischaemia (96). In the context of cardiac surgery, it is the results of intermittent aortic cross-clamping, intermittent or continuous administration of cardioplegic solutions, cross-clamp fibrillation or a combinations of these procedures (83). Myocardial IRI has been recognised as the most relevant potential cause of PMI in patients undergoing cardiac surgery (83) and has therefore been the subject of extensive experimental and clinical investigations conducted throughout the last few decades. These will be discussed in details in the next section.

It is often difficult to distinguish PMI from MI associated with CABG surgery, termed type 5 MI, and this is due to the fact the two processes have some pathogenetic factors in common and can be identified with similar diagnostic tools (97). Type 5 MI is defined by cardiac biomarkers rise more than five times the 99th percentile of the normal reference range during the first 72 hours following CABG, associated with the appearance of new pathological Q-waves or new left bundle branch block (LBBB), or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium (97). Similarly to PMI, Type 5 pathogenesis is related to multiple potential factors leading to peri-procedural necrosis, including direct myocardial trauma from sewing needles or manipulation of the heart,

coronary dissection, global or regional ischaemia secondary to inadequate cardiac protection, microvascular events due to reperfusion, myocardial damage induced by ROS generation, or failure to reperfuse areas of the myocardium that are not subtended by graftable vessels (98-100).

1.3.1. Identification of PMI following cardiac surgery

As with type 5 MI, a variety of diagnostic approaches have been developed to identify PMI during cardiac surgery and correlate this with prognostic significance: clearly one of the most important strategy is a close monitor of any potential ECG changes, which might be suggestive of myocardial ischaemia. During the last few decades, also highly sensitive ***imaging tests*** have been developed and include:

- contrast enhanced cardiac magnetic resonance imaging (CMR), which has demonstrated a diminished degree of LV ejection fraction (LVEF) reduction in patients undergoing off-pump cardioplegia compared to those undergoing cardioplegia (101);
- tissue Doppler echocardiography, which has showed an association between PMI measured by troponin I (TnI) rise and reduced right ventricular (RV) velocities following paediatric surgery (102);
- in addition, several radionuclide tracers, including thallium-201, technetium-99m MIBI, tetrofosmin and ^{18}F2-fluorodeoxyglucose (FDG) allow viable myocytes to be imaged directly (103-105), although the relatively low resolution of the images can limit the detection of small areas of infarction (106). In particular, ECG-gated imaging provides a reliable assessment of myocardial motion, thickening, and global function (107, 108).

Crucially, these imaging modalities often fail to identify subtle degrees of myocardial injury which may have instead prognostic relevance (109) and furthermore, their use in research and clinical practice is limited by their significant financial burden and the more accessible availability and sensitivity of serum cardiac biomarkers. The emergence of cardiac biomarkers has made considerable advance in understanding the occurrence of myocardial injury during cardiac surgery, and its prognostic significance (**Table 1.4**).

In particular, the GUARDIAN and ARTS studies first and Brener and colleagues showed that post-operative CK-MB levels greater than 10 times the upper limit of normal (ULN), were a positive predictor of mortality at 6-months and one-year (110, 111). However, it was with the introduction of cardiac troponin that the diagnosis of PMI in cardiac surgery and in general of MI has been truly revolutionised. Cardiac troponin isoforms, such as troponin T, I or C (TnT, TnI, TnC) are integral part of the thin filaments of myocite cytoskeleton: TnT and TnI are not expressed in skeletal muscle, whereas TnC is also present in smooth muscle, which makes the former specific markers of myocardial injury (112). Following myocardial injury, there is first a troponin release within 3-5 hours from membrane destruction, and a second release on the 5th subsequent day due to contractile apparatus damage (113). Eigel et al were the first to look at the prognostic implications of cardiac troponin rise following cardiac surgery: in a study of 540 patients undergoing CABG surgery, they showed that post-surgical increases of cTnI concentrations were associated with worse clinical outcomes and that a cut-off at cTnI>0.495ng/L was a strong predictor of adverse prognosis. TnI has also been demonstrated to be superior to CK-MB in identifying PMI in patients undergoing CABG surgery as it offers greater accuracy and a higher sensitivity, although more recently this finding has been quite controversial (82): in a

retrospective analysis of 545 patients undergoing CABG surgery, Muehlschlegel and co-workers (82) demonstrated that cTnI was the strongest predictor of 5-year mortality compared to ECG and CK-MB. These findings were subsequently validated by other studies confirming that both TnT (114-117) and TnI (80, 118) rise following cardiac surgery is associated with worse short and long-term clinical outcomes (77, 82). More recently, a high-sensitivity TnT assay (hsTnT) has been introduced to increase TnT detection and is between 1000 and 10000 times more sensitive than the original first generation assays (Singulex high sensitivity TnI): it therefore has a high negative predictive value in the diagnosis of ACS and myocardial injury in general, allowing an improvement of diagnosis certainty and timing at time points earlier than 10-12hrs. HsTnT 99th percentile of ULN is 0.014 ng/L with a 10% coefficient of variation of 0.03 ng/L and a limit of detection of 0.0053 ng/L. However, the disadvantage of its sensitivity is the frequent occurrence of hsTnT rise also in the context of conditions other than myocardial injury, particularly inflammatory-infective illnesses and renal impairment (112). It is relevant to note that hsTnT assay was used at the single centre where we conducted our study on remote ischaemic preconditioning (RIPC) using multi-limb IR, as well as at the 28 participating centres of the ERICCA trial.

Table 1.4. Major studies investigating the prognostic value of cardiac biomarkers in the setting of cardiac surgery

Study	Patient number and clinical setting	Cardiac biomarker	Outcome
Brener(119) 2002	3812 CABG	CK-MB	> 10 x ULN = independent predictor of mortality
Eigel (120) 2001	540 CABG	Troponin-I	> 0.495ng/L = cut-off adverse outcomes prediction
Lasocki (121) 2002	502 CABG or valve surgery	Troponin-I	>13ng/ml independent predictor of in-hospital mortality.
Fellahi (80) 2003	202 CABG	Troponin-I	>13ng/ml = increased 2-year mortality risk
Katherisan (77) 2004	136 CABG	Troponin-T	> 1.58ng/ml = 1-year mortality rate predictor
Lehrke (78) 2004	204 CABG	Troponin-T	>/=0.46ug/L = 4.9-fold increase risk of mortality
Paparella (122) 2005	230 CABG	Troponin-I	> 13ng/l = predictor of in-hospital mortality, not of 2 years outcome
Bottio (123) 2006	520 Correction of congenital heart disease	Troponin-I	>35µg/L = no prognostic significance at 12 months
Mildh (124) 2006	1001 Paediatric cardiac surgery	Troponin-I	> 5.9µg/L = predictor of death
Fellahi (115) 2008	184 CABG or AVR	Troponin-I	serial troponin-I release and single 24-hr measurement were equally good as predictors of in-hospital outcome
Buse (125) 2009	741 CABG	Troponin-T	> 0.1µg/L = predictor of 12-month mortality
Nesher (118) 2008	1918 CABG and/or valve surgery	Troponin-T	>0.8µg/L = increased MACCE
Muehlschlegel (82) 2009	545 CABG	Troponin-I	Compared with ECG and CK-MB, troponin-I was strongest predictor of 5 year mortality
Mohammed (79) 2009	847 CABG	Troponin-T	Levels associated linearly with length of stay and ventilator hours and with death, death or heart failure, death or need for vasopressor and the composite of all 3
Van Geene (117) 2010	938 CABG or valve surgery	Troponin-I	> 4.25nl/L = cut-off as in-hospital mortality predictor

CABG=coronary artery bypass graft; AVR=aortic valve replacement; ULN=upper limit of normal; CK=creatine kinase; MACCE=major adverse cardiac and cerebrovascular events

1.4. Myocardial Ischaemia-Reperfusion Injury

As previously described, myocardial IRI defines the phenomenon by which the restoration of blood flow to an organ or tissue following a prolonged period of ischaemia can paradoxically lead to this organ or tissue injury (96, 126). This process has been extensively investigated in the heart and more recently has also been observed in other organs or tissues, such as kidneys, lungs, liver, brain, intestine, skin, skeletal muscle and ovaries, as we will discuss later. In the context of acute MI, successful restoration of reperfusion through thrombolytic therapy or primary PCI can itself induce cardiomyocyte death and increase infarct size. In animal models, it has been observed that IRI could account for up to 50% of the final infarct size (96, 127, 128). Manifestations of myocardial IRI may manifest include (129):

1. Reperfusion arrhythmias.
2. Myocardial stunning.
3. No-reflow phenomenon.
4. Lethal ischaemia-reperfusion injury (IRI).

1.4.1. Ischaemic Injury

With the ischaemic insult and the depletion of oxygen and nutrients, oxidative phosphorylation is significantly reduced with progressive ATP depletion and activation of the anaerobic glycolytic pathway, therefore leading to intracellular accumulation of lactic acid and loss of sodium and potassium (130). This subsequently causes gradual cellular swelling and increases cellular acidosis thus activating the sodium-hydrogen exchanger, with further sodium accumulation and initiation of reversed activity of the sodium-calcium exchanger. In physiological conditions the latter favours sodium import

and calcium export in order to constantly regulate intracellular calcium concentration. However, in the context of an ischaemic insult causing sodium accumulation, the sodium-calcium exchanger removes sodium from the cell and imports calcium into it (131). At the same time, in an attempt at ensuring ATP production, the cell uses fatty acids from cellular and mitochondrial membranes, therefore leading to loss of membrane integrity with further accumulation of sodium and calcium inside cytoplasm and mitochondrial matrix. This then worsens the loss of cellular components and contributes to a transition from a potentially reversible phenomenon into an irreversible process leading to initiation of cell necrosis (132). The loss of mitochondrial membrane integrity also induces intracellular release of enzymes including cytochromes, caspase and protheolytic enzymes, ultimately responsible for cellular apoptosis and autophagy (130, 132).

1.4.2. Reperfusion arrhythmias

In the clinical setting, these occur particularly following thrombolysis, PPCI or cardiac surgery and may manifest as an accelerated idioventricular rhythm (133). The pathogenesis of reperfusion arrhythmias is linked to the loss of permeability of mitochondrial membrane, which leads to destabilisation of the action potential across the cell membrane thereby increasing the susceptibility for the initiation of arrhythmias (133).

1.4.3. Myocardial stunning

This describes the myocardial dysfunction occurring further to an ischaemic injury and blood flow restoration. It is a transient, reversible phenomenon caused by persistent anaerobic metabolism following reperfusion and oxidative stress (134).

1.4.4. No-reflow phenomenon or microvascular obstruction

This may represent the consequence of platelet and complement cascade activation initiated by the ischaemic insult and consists of severe dysfunction of the resting blood flow in the microvasculature within the ischaemic area (135). Terminal complement cascade components cause direct injury to endothelial cells with platelet activation and reduced endothelial production of nitric oxide (NO), subsequent vasoconstriction, diminished microvascular perfusion and tissue necrosis (136).

1.4.5. Lethal ischaemia-reperfusion injury

Lethal IRI refers to the cell injury and death resulting from the restoration of blood flow to a tissue or organ subjected to a prolonged period of ischaemia and only reversibly injured by the ischaemic event (96).

A significant number of potential mechanisms have been implicated in the pathogenesis of lethal IRI and these have been the subject of extensive experimental studies, however the translation of these findings from animal models to the clinical setting has been often disappointing (96) thereby raising the question of potentially significant disparities between the two settings (96). Therefore further laboratory and clinical studies are needed in order to more extensively explore these fascinating mechanisms and identify agents able to reduce myocardial IRI and improve clinical outcomes in patients with known IHD and undergoing revascularisation (137). Mechanisms implicated in the pathogenesis of lethal IRI include:

1. **Oxygen paradox.** Restoration of oxygenation to an ischaemic area leads to activation of xanthine oxydase, neutrophils, cyclooxygenase, lipoxygenase, and to catecholamine oxidation, with subsequent ROS production, cell injury and death (138, 139).

2. **pH Paradox.** Reperfusion enables cell washout of the previously accumulated lactic acid, with rapid restoration of intracellular pH, and in concomitance with the sodium-hydrogen exchanger and the sodium-bicarbonate exchanger activation: this paradoxically contributes to lethal IRI by inducing the mPTP opening and myocyte hypercontracture (140).

3. **Calcium paradox.** Following restoration of normal pH, an increased intracellular calcium accumulation occurs as a consequence of the reversed activity of the calcium-sodium exchanger due to sodium-hydrogen exchanger activation, and to cell, sarcolemmal and mitochondrial membrane permeability loss (141), with disproportionate myofibrils contracture and subsequent myocyte hypercontracture (142), damage to myofibrils and other cytoskeletal components, and loss of intercellular junctions (140).

4. **Inflammation**. Cytokines and activated complement components released by the damaged myocardium induce neutrophil activation (143), with subsequent production of proteases and elastases, which then contribute to cell destruction and death (144).

In summary, it is clear that the understanding of the mechanisms underlying IRI can have an enormous impact on treatment of IHD and in general of conditions where IRI can occur. One of the potential crucial aspects of managing patients presenting with acute MI is, besides the prompt restoration of blood flow to the ischaemic areas, the prevention or at least the limitation of IRI and, in this regard, the seminal paper by Murry et al (145) provided a new potential strategy to limit infarct size and heralded the era of ischaemic preconditioning.

1.5. Ischaemic Preconditioning

The myocardium possesses innate physiological adaptive processes enabling it to become more resistant to subsequent lethal ischaemic injury. These include the development of coronary collaterals vessels, myocardium stunning and hibernation, and crucially ischaemic preconditioning (IPC) and postconditioning (IPost) (146). The concept of IPC was first introduced with the pioneering work by Murry et al in 1986 (145), who reported a 75% infarct size reduction in dogs subjected to four-five minutes episodes of regional myocardial ischaemia, each followed by a five-minute period of reperfusion, and prior to a prolonged period of ischaemia: the protection given by brief episodes of sub-lethal ischaemia prior to a lethal index ischaemic insult was then confirmed in a significant number of studies (147-151), and was also demonstrated in other organs, including kidney (152), brain (153), lung (154), liver (155) and skeletal muscle (156). Crucially, following an ischaemic event, studies have showed a first period of protection, called “first window of preconditioning” or “early” or “classic” preconditioning which occurs immediately after the index insult and usually wanes off after 1-2 hours (157, 158), and a delayed and less protective “second window of preconditioning” after 12-24 hours, lasting up to 72 hours (159-162).

1.5.1. Mechanisms of IPC

The mechanisms underlying IPC involve the production of mediators, which following the binding with specific receptors, activate intracellular transduction pathways able to induce end-effectors ultimately responsible for cell protection.

Potential **mediators** of IPC have been identified in adenosine (163-165), opioids (166-170), acetylcholine (171, 172), catecholamines (173), angiotensin II (174), bradykinin

(175), endothelin (176), and ROS (177-181). **Cell membrane receptors** include: G_i protein-coupled receptor (GPCR), for adenosine (165), bradykinin (175), opioids (168), angiotensin II (174), catecholamines (173), endothelin (176), urocortin, adrenomedullin and glucagon-like peptide (182); growth-factor receptors, for insulin, insulin-like-growth factor, transforming growth factor, fibroblast growth factor, granulocyte colony stimulating factor, erythropoietin and adipocytokines (182); and ligand specific receptors for atrial natriuretic peptide (ANP). In addition other non-receptor mediated pathways involved in IPC triggers have been described, such as mechanical stimuli such heat and stretch, and substances such as metformin, statins and volatile anaesthetics (182).

Following receptors activation, an intriguing cascade of signal **intracellular transduction pathways** occurs and involves the activation of pro-survival protein kinases, such as the Reperfusion Injury Salvage Kinase (RISK)(182, 183), Survivor Activator Factor Enhancement (SAFE)(184), which ultimately lead to the inhibition of the opening of mitochondrial permeability transition pore (mPTP) (185, 186), the most-important **end-effector** of IPC, thereby producing anti-necrotic, anti-apoptotic and anti-autophagic effects and reducing cardiomyocyte death (182, 187). MPTP is a non-specific high conductance channel situated in the inner mitochondrial membrane, which remains closed during ischemia and opens in the first few minutes of reperfusion (188). It plays a major role in IRI as its opening induces: 1) increased water and solutes influx into the mitochondria, with subsequent rupture of outer mitochondrial membrane and release of intermembrane cytochrome C and initiation of cell apoptosis and necrosis (189, 190); 2) uncoupling of oxidative phosphorylation, leading to ATP hydrolysis, progressive ATP depletion, collapse of the mitochondrial membrane potential and cardiomyocyte death (189, 190). MPTP comprises the

complex F_OF_1 ATP synthase (or complex V), the rotary enzyme able to synthetise the vast majority of ATP on the inner mitochondrial membrane (191), and to bind magnesium and ADP/ATP in the presence of low calcium concentrations (191): however, the high calcium levels subsequent to an ischaemic event lead cyclophylin-D in the mitochondrial matrix to bind with the lateral stalk of complex V, thereby causing a conformational change ultimately responsible for mPTP formation. Crucially, cyclosporine-A inhibits mPTP opening by preventing cyclophylin-D from binding F_OF_1 dimers (191, 192) and has been demonstrated to induce LV function recovery, ATP preservation, and MI reduction when given at reperfusion (193): it is therefore understandable that mPTP has soon become an important target of a significant number of both experimental and clinical studies in the search for the specific mechanism able to prevent mPTP formation and subsequent myocardial cell death (194).Two other major end-effectors are the sodium-hydrogen exchanger, which reduces oncotic swelling of the cells and inhibits sodium-calcium exchanger thereby reducing intracellular calcium accumulation, and the gap junctions which favour electrical coupling of cardiomyocytes and transport of active substances (195).

1.6. Ischaemic Postconditioning

A crucial aspect of the cardioprotective effects of IPC is the need to apply the preconditioning stimulus prior to the index ischaemic insult. However in clinical practice this is not applicable, as the onset of ischaemia in acute settings such as an MI cannot be anticipated. In this regard, the finding that the application of the conditioning stimulus after the onset of the index ischaemic insult (IPost) is able to

protect the heart from lethal IRI (196), provided a new potential strategy to reduce lethal IRI. Intriguingly, further studies demonstrated that IPC and IPost may share similar mechanisms (197-200). However, both IPC and IPost require an invasive stimulus to be applied to the heart in order to confer cardioprotection, which has significantly limited their clinical application: the discovery that the heart can be protected by brief episodes of ischaemia and reperfusion applied to a distant organ or tissue prior to a period of sustained ischaemia, offered the potential of an innovative strategy for enhancing cardioprotection (201).

1.7. Remote Ischaemic Preconditioning

Remote ischaemic preconditioning (RIPC) describes the phenomenon by which brief episodes of sub-lethal ischaemia and reperfusion to one organ or tissue distant from another organ or tissue, are able to reduce IRI in this organ/tissue (201). This intriguing concept was first introduced by Przyklenk et al (201) with an ingenious experiment sought to determine whether in anaesthetised dogs brief occlusions in one myocardial vascular bed could also limit infarct size and/or attenuate contractile dysfunction in remote “virgin” myocardium subjected to subsequent sustained coronary occlusion. Dogs were subjected to four episodes of 5-minute circumflex (Cx) occlusion and 5-minute reperfusion, followed by 1 hour of sustained LAD occlusion and 4.5 hours of reflow. Infarct size was significantly reduced (63%) in Cx preconditioned dogs compared to controls, proving that virgin myocardium could be protected from subsequent sustained coronary occlusion by brief episodes of ischaemia in a distant or “remote” vascular bed. They also suggested that this

protective effect could be mediated by “factor(s) activated, produced, or transported throughout the heart during brief ischemia/reperfusion” (201). This was therefore the first demonstration at the same time of the existence of both RIPC and its potential underlying mechanism: however similar results could not be reproduced in a study by Nakano and colleagues (202), who subjected rabbit hearts to IPC followed by explantation of the hearts and 30 minutes of global ischaemia with two hours of reperfusion: only the areas previously receiving IPC could be protected whereas those distant or “remote” had no benefit: this led to the conclusion that RIPC could be species or protocol-specific but also subsequently to the concept that intact humoral or neural mechanisms are required for RIPC to occur (203).

From these models of intra-myocardium or *intra-organ RIPC*, subsequent experimental studies have brought to the discovery that cardioprotection can also be elicited when the RIPC stimulus is applied to organs or tissues different from the heart, including kidneys (204-209), intestine (205, 210-216), skeletal muscle (217-230) but not brain (231-233), therefore leading to the concept of *inter-organ RIPC.*

Importantly, the recent discovery that cardioprotection can be induced by remotely preconditioning skeletal muscle/hind limbs led to the vast application of this type of RIPC stimulus to human studies: Birnbaum et al (217) were the first group to apply RIPC to an animal model hind limb. In a seminal study, they randomised anaesthetised rabbits to 30 minutes of waiting period (controls), or 55% to 65% reduction of femoral artery stenosis, or electrical stimulation of the gastrocnemius muscle, or stenosis plus stimulation: this was followed by 30 minutes of coronary artery occlusion and 4 hours of reperfusion. They found that the ratio of infarct size/risk zone was significantly smaller in the stenosis plus stimulation group compared to control, stenosis, and stimulation groups, thereby concluding that the combination of

muscle stimulation and reduction of femoral arterial blood flow but not muscle stimulation alone or flow restriction alone could reduce MI size. An important implication of this study was that by reducing arterial blood to 55-65% and inducing at the same time a demand-supply imbalance with the rapid pacing of the gastrocnemius muscle, the “transport” of a mediator of RIPC would be facilitated thereby obviating the need for a reperfusion phase. From Birnbaum’s innovative idea, Oxman and colleagues (218) were the first to apply a non-invasive method of preconditioning stimulus. In rat models, they used a tourniquet to induce 10 minutes ischaemia and reperfusion of hind limb and found a significant reduction of the incidence of reperfusion tachyarrhythmias in the preconditioned group. Whilst a significant value of this study was to suggest a potential mediator for RIPC, its most important contribution to the further progress in understanding the mechanisms of preconditioning was the application of a non-invasive method of cardioprotection, which would understandably find wide application to humans in a number of different clinical settings.

1.7.1. Mechanisms of RIPC

A significant number of experimental and clinical studies has been conducted throughout the decades in order to identify the mechanistic pathway through which RIPC can protect an organ or tissue “at a distance”. These have been focused on three crucial targets (234):

1) the mechanisms triggered by the preconditioning stimulus in the remote organ/tissue subjected to IR;
2) the transmission pathway of the protective stimulus from the organ or tissue where it is applied to the organ or tissue that is ultimately protected;
3) the cellular mechanisms through which this stimulus is able to confer organ or

tissue protection against a sustained lethal ischaemia.

1.7.1.1. Generation of the cardioprotective stimulus in the preconditioned remote organ/tissue

Despite the significant amount of research in this filed, we are still far from the identification of the specific mechanisms triggered by the preconditioning stimulus at the distant organ or tissue. However, it is well known that transient periods of IR trigger the production and subsequent release of various substances from the temporarily ischaemic tissue (234): these present an inter-species variability but also, and intriguingly, an intra-species variability (235), which could also be related to the different types of mediators and mechanisms involved at the different sites of the stimulus (236).

In the context of cardioprotection induced by skeletal muscle IR, **opioids** have been found to play a crucial role: Patel et al (211) demonstrated for the first time that the protective myocardial effect given by RIPC in rats could be abolished by the opioids antagonist naloxone: this finding was also by subsequent studies later (220, 237-239), which also led to the identification of δ1-(220, 238) and k-(239) opioid receptors ad key components of the intracellular transmission of the preconditioning stimulus.

Importantly, Chen et al (224) demonstrated that **NO** could represent another important mediator of limb RIPC in rats where the protective effects of hind limb IR was abolished by NO-synthase antagonist L-nitro-arginine methyl ester (L-NAME) and similarly Shashid and colleagues (221) proved that femoral artery ischaemia-RIPC was mediated by a combination of NO production, mito-KATP channel activation and **ROS** release. Subsequently, Chen and co-workers (240) confirmed the involvement of ROS in skeletal muscle IR by showing that the protective myocardial effects in rats

were associated with increased activity of superoxidase dismutase and glutathione peroxidase and could be abolished by mercaptopropionyl-glycine, a free radical scavenger.

In the context of mesenteric ischaemia, opioids (211, 241) and **cannabinoids** (242, 243) have been found to be implicated in cardioprotection, with particular evidence in favour of endocannabinoid CB2 receptors but not endocannabinoid CB1 receptors. The neurotransmitter **CGRP** has also been found be involved in both early and late preconditioning (213, 244) following the intriguing experiments by Tang and colleagues (213) who observed reduced CGRP levels and abrogation of RIPC in rabbits following the administration of capsaicin, which cause nerve depletion of CGRP. As with studies involving adenosine, it has also been proved that the administration of hexametonium could abolish preconditioning induced by CGRP and this was correlated with diminished PKC-ε activation (245). Similarly, the activation of intracellular PKC-ε (215) and the abrogation of cardioprotection with the administration of **bradykinin** B2 receptor antagonist HOE140 and hexametonium followed by reactivation of RIPC further to bradykinin infusion (212) proved that bradykinin may also be implicated in RIPC.

Interestingly, **adenosine** has been found to be involved in cardioprotection by renal ischaemia, both with “neural transmission” at remote level and translation of neural signals in the preconditioned organ (210), as demonstrated by:

- the abrogation of the protective effects in the presence of adenosine receptor antagonists (206, 207);
- the increased blood level of adenosine in preconditioned rabbits (207) and mice (233);
- the resistance to cerebral RIPC in adenosine receptor (A1R) knockout mice (233);

- the attenuation of renal efferent nerves discharge following preconditioning by adenosine receptor antagonist 8-sulphophenyltheophylline (8-SPT) (246);
- the reoccurrence of cardioprotection following intra-mesenteric infusion of adenosine further to the addition of hexametonium and 8-SPT (247) and infra-femoral infusion of adenosine further to femoral never section and the addition of 8-SPT (248).

These mediators, once released following the transient IR stimulus from the remote organ or tissue, are then able to “transfer” the signal to the myocardium as well as other distant organs or tissues including kidney, liver, lungs, brain, skin, ovaries and gastro-intestinal system. The specific mechanisms responsible for the “transmission” of the preconditioning stimulus to the targets organ/tissue have not yet been fully determined, however three main pathways have been identified with evidence in favour of each of them (234):

1. neural pathway;
2. humoral pathway;
3. systemic inflammatory response.

1.7.1.2. Signal transmission to target organ/tissue

In 1996, Gho and co-workers (205) made the crucial discovery that cardioprotection could be induced by applying the preconditioning stimulus with brief episodes of anterior mesenteric artery or left renal artery occlusion and identified two fundamental aspects of the potential underlying RIPC mechanisms:

1) transient but not continuous mesenteric occlusion enhanced cardioprotection,

suggesting that a period of washout was necessary for a putative humoral factor to be produced in the ischaemic tissue or organ and to be then transferred to distant organs or tissues;

2) the administration of the ganglion blocker hexametonium abolished the cardioprotective effects of transient mesenteric ischaemia, therefore indicating the possibility of an involvement of also a neuronal mechanism.

Over the years, the scientific community has produced studies in favour of both the humoral and the neuronal theory but we are still quite far from the identification of the exact mechanism and more importantly, of the agent ultimately responsible for remote protection. The **neural theory** postulates that mediators produced in the "distant" ischaemic territory activate local afferent pathways first and efferent pathways then, which are then responsible for the transmission of the remote preconditioning stimulus to the target organ/tissue (234). Conversely, the **humoral theory** hypothesises that that the transient ischaemic insult in the remote organ/tissue leads to the local production of substances and their subsequent release into the systemic circulation, through which they are then able to reach and "protect" the target organ/tissue (234): this was consolidated by the finding that a period of reperfusion of the remote organ was necessary following brief ischaemia, suggesting that the reperfusion phase was required to 'washout' a substance or humoral factor generated by the transiently ischaemic territory, to be then transported through the vascular system to the heart (234). Birnbaum et al (217) hypothesised that cardioprotection could be induced via a humoral pathway by rapid pacing of the gastrocnemius muscle in the rabbit which caused partial femoral artery occlusion and therefore in the absence of the reperfusion phase. Subsequently, the humoral theory was further confirmed by Kristiansen (226) and Kostantinov (225), who found that previously preconditioned explanted and

therefore denervated hearts could be protected against prolonged ischaemia in rats (249) and pigs (225, 249). Similarly, Wang et al (214) demonstrated that hexametonium did not abolish cardioprotection induced by mesenteric ischaemia in contrast with the finding from Gho and co-workers (205), and Dickson's groups (237, 241, 250, 251) went further on to the "search" for the humoral factor, suggesting that this could involve norepinephrine as a potential mediator of RIPC (251). To date we have not yet identified such a mediator: crucially, it has been identified as a thermolabile, hydrophobic substance with a molecular weight between 3.5 KDa (252) and 15 kDa (230, 253). Other potential "humoral" mediators of RIPC have been identified in erythropoietin (208), hypoxia-inducible factor (HIF) (209), stromal derived factor-1 (254) and angiotensin-I (255) although their exact role has yet to be clarified.

Importantly, at the same time, other relevant studies strengthened the neuronal theory confirming Gho's observation that cardioprotection could be blocked by ganglion antagonist hexametonium (210, 212, 215) and by proving that an intact renal nerve was essential in cardioprotection by renal preconditioning (246), although the role of an intact femoral nerve was more controversial (228, 248). In addition, nicotinic receptors antagonists and reserpine, a neurotransmitter uptake inhibitor at the level of the synaptic vesicle, have been demonstrated to interfere with RIPC (218, 256) and activation of the dorsal motor nucleus of the vagus nerve has been showed to induce cardioprotection even in the absence of the remote preconditioning stimulus in the skeletal muscle (257). Furthermore, substances such as adenosine (258), bradykinin (212) and CGRP have been associated with the neuronal mechanisms of RIPC, as findings suggest that their production is increased in the preconditioned organ and that they activate afferent neural pathways, which are ultimately responsible for the cardioprotective effect. Crucially some of these substances activate intra-cellular

preconditioning pathways through the binding and stimulation of membrane receptors, such as A1, BK2, δ1-opioid, k-opioid and angiotensin 1-receptors, which all belong to the GPCR family and have already been described in the previous sections (234). An important implication of this is that IPC, RIPC and IPostC may share intracellular pathways in order to ultimately induce cardioprotection (234). Furthermore, Jensen et al (259) suggested that MI size in isolated naïve rabbit hearts could be reduced by the plasma dialysate obtained by RIPC-treated patients with or without DM and without sensory neuropathy but not by RIPC-treated diabetics with sensory neuropathy: this suggested firstly a crucial interaction between neural (vagal) and humoral pathways and secondly a sequential activation of mechanisms given by adenosine production in the preconditioned limb, sensory neural pathway activation though NO and brainstem dorsal nuclei stimulation, although the process through which the signalling is transmitted from the brainstem to the heart remains unclear (257, 260).

In addition, the third potential mechanism potentially able to explain RIPC-signal transmission is the **systemic anti-inflammatory response**: this was first hypothesised by Peralta and colleagues (261), who found that hepatic RIPC could produce an anti-inflammatory effect through modulation of myocardial gene transcription profile with P-selectin up-regulation inhibition, ultimately resulting in reduced neutrophil migration and oxidative stress and therefore in an anti-inflammatory and anti-apoptotic effect. This concept was subsequently confirmed by further animal studies involving different organs including lungs (262, 263), stomach (244), and myocardium (264) but also in human trials (265).

In conclusion, it is important to appreciate that despite significant efforts to elucidate these mechanistic pathways, none of these has been fully and exclusively accepted and it is more likely that no single mechanism is uniquely responsible but

rather that several complementary pathways coexist, interact with each other and are therefore not mutually exclusive (234).

1.7.1.3. Intracellular signal transduction pathways and end-effectors of RIPC

Experimental studies have showed that PKC activation may mediate the protective effects of RIPC and that cardioprotection could be abolished by the administration of PKC blockers (214, 215, 228, 245): the isoform PKC-ε may be particularly implicated in the signal transduction following bradykinin BK2 (215) and CGRP (245) receptors stimulation. Similarly, mito-K_{ATP} channel has a role in the signalling pathways, as demonstrated by the abrogation of RIPC with specific blockers such as 5-hydroxytryptamine (5HD) blocker (206) and glibenclamide (225, 226). Crucially, Loukogeorgakis et al (266) demonstrated that the protective effects of both remote post-conditioning (RIPostC) and RIPC on endothelial function in humans was mediated by mito-K_{ATP} channel and that, similarly to animal models, this could be blocked by the administration of glibenclamide. Additionally, as with IPC, ROS might also have a significant role in transduction of the remote preconditioning stimulus (220) and its function might be closely correlated to that of NO and mito-K_{ATP} channel (221). NO has itself been showed to be involved in RIPC as demonstrated by both the induction of preconditioning following the administration of NO donors in rats (267) and the observation that such cardioprotection could be abolished by NO inhibitor L-NAME (224, 267), whereas further experimental studies particularly in mice have demonstrated the predominant role of NO in the delayed rather than the acute phase of preconditioning (214, 216, 225, 232). Crucially, the RISK pathway is the family of pro-survival protein kinases the main components of which are PI3-K/Akt and Erk

(1/2), may play an essential role in the intracellular mechanisms of IPC (183, 268-273), IPostC (274), but also in RIPC as recent studies have demonstrated that:

- inhibition of p38, Erk 1/2 and JNK 1/2 abrogates cardioprotection following mesenteric preconditioning (275),
- activation of p38 can mediate rat adipocutaneous flaps induced by limb IR (276),
- p42/44 stimulation is essential for the cardioprotective effects of limb preconditioning both in rabbits and humans (229).

Another important similarity amongst these cardioprotective mechanisms is represented by the end-effector mPTP: in the context of RIPC, mPTP has been showed to represent the likely downstream target of both mito-K_{ATP} activation (277) and RISK pathway (272, 273). Cao et al (278) demonstrated that in anesthetised male Sprague-Dawley rats, cardioprotection could be induced by three cycles of 5 minutes of right femoral artery occlusion followed by 5 minutes of reperfusion and that these effects were attenuated by the mPTP activator atractyloside, whereas administration of the mPTP inhibitor cyclosporin A decreased the effect of IR, thereby demonstrating that the inhibition of mPTP opening is a crucial aspect of cardioprotection by RIPC.

1.7.2. Clinical applications of RIPC

Following the pioneering discovery that the myocardium could be protected against IRI by transient hind limb IR in animal models (217, 218), the clinical potential of RIPC soon became very clear. Two major clinical properties of RIPC allowed its rapid translation into clinical research:

1. Its *feasibility*: the seminal finding that a non-invasive preconditioning stimulus could be applied to human volunteers with inflation/deflation of a simple blood pressure cuff inducing transient limb IR (222), heralded the application of RIPC into different clinical settings, which rapidly increased throughout the years and is yet intriguingly due to further expand to new contexts not so far being fully investigated.
2. Its *flexibility*: the main limitations of both IPC and IPost are the requirement for the stimulus to be applied at a specific time, respectively prior to the ischaemic phase and at the onset of the reperfusion phase, and directly to the heart; conversely, the remote conditioning stimulus can be applied prior to (RIPC), after the onset of (RiPerC) or at the end of (RiPostC) ischaemia and to an organ/tissue distant or "remote" from the heart (274).

Crucially, the first clinical trial to investigate the effects of RIPC on myocardial protection was by Gunaydin et al (279) in patients undergoing CABG surgery: the RIPC stimulus was applied by simply using a tourniquet around the patients' right upper arm for 3 minute, followed by 2 minutes of tourniquet release (a cycle which was repeated twice). Markers of PMI including CK-MB and lactate dehydrogenase (LDH) were measured before CPB, prior to declamping of the aorta and 5 minute after declamping of the aorta. Only LDH levels at the second time point were significantly higher in the preconditioned patients with no other statistically significant difference between the groups: however this study was clearly underpowered as it only included 8 patients. Subsequently, MacAllister's group (222) characterised a simple non-invasive RIPC protocol in healthy human volunteers, which was then extensively used in the subsequent clinical trials on RIPC: in this pioneering study, three cycles of 5-minute ischemia and reperfusion of the upper limb with a simple blood pressure cuff

inflation to 200 mmHg and deflation were applied prior to prolonged ischaemia in the contralateral arm subjected to blood pressure cuff inflation to 200 mm Hg for 20 minutes, followed by deflation. This resulted in an increased response to acetylcholine in the forearm subjected to prolonged ischaemia as demonstrated by venous plethysmography. The application of this simple, non-invasive and virtually risk-free intervention heralded a new era of research of the potential benefits of RIPC delivered by transient limb IR in the setting of cardiac surgery in the first instance, and more recently also in different contexts, including non-cardiac surgery, elective or primary PCI (280). Importantly, the remote conditioning stimulus applied in the setting of PPCI, is more correctly defined as **remote ischaemic perconditioning** (RIPerC) as the transient limb IR induced by inflation and deflation of the blood pressure cuff takes place after the onset of the ischaemic insult (274). Conversely, the stimulus is termed **remote ischaemic postconditioning** (RIPostC) when applied at the time of myocardial reperfusion (144).

1.7.2.1. RIPC and Cardiac Surgery

Following the original study by MacAllister's group (222), Cheung et al (281) were the first apply the concept of RIPC with limb IR to the clinical setting: in a pioneering trial involving 17 children undergoing repair of congenital heart defects, they demonstrated that RIPC, induced by 4 cycles of 5 minute inflation of a blood pressure cuff applied to the lower limb, followed by 5 minutes of deflation, reduced postoperative levels of cTnI, inotropic requirements at 3 and 6 hours, and airway resistance.

Our group (282) showed for the first time that adult patients undergoing elective CABG surgery and receiving three-5 minutes cycles of upper arm IR sustained a significantly lower magnitude of PMI than control patients. Since these ingenious applications, RIPC with limb IR has been extensively investigated in a significant number of clinical studies in the setting of CABG surgery alone, valve surgery alone or a combination of the two and in the context of corrective paediatric surgery for congenital heart disease **(Table 1.5)**: importantly, the majority of these trials have confirmed the cardioprotective effects of RIPC, however more recently a number of RCTs have failed to demonstrate any significant beneficial effects. The potential reasons for this will be extensively elucidated in chapter 3, however here we intend to briefly explain the limitations of these studies and the possible causes for the negative results in order to then clarify the rationale of our two studies.

1.7.2.1.1. "Drawbacks" of clinical trials on RIPC in cardiac surgery

A first crucial consideration emerging from the analysis of the RCTs so far conducted to determine the effects of RIPC in the context of cardiac surgery is represented by the highly difficult translation "from benchmark to bedside" and therefore from the setting of experimental studies to that of human trials. The clinical context that most closely matches the experimental model is the patient undergoing PPCI for STEMI, which is secondary to acute coronary artery occlusion: similarly, animal models are subjected to myocardial IRI by direct ligature of the coronary artery. Conversely, the majority of the clinical studies on RIPC have been conducted in the context of elective cardiac surgery, where also the magnitude of myocardial injury is relatively low and it is therefore possible that the further beneficial effect provided to these patients by RIPC might be too small to be have significant relevance in the

studies evaluated (280). Moreover, animal models present either no pre-existing disease or experimentally reproduced conditions, whereas the vast majority of patients with CAD have multiple co-morbidities, such as hypercholesterolemia, hypertension and above all diabetes, which may have a crucial impact on RIPC-induced cardioprotection (this will be discussed in details in chapter 6). In addition, animal models are not on concomitant pharmacological therapy and, conversely, patients enrolled into RCTs are often on various medications, including insulin, atorvastatin nicorandil, clopidogrel, cangrelor, and may additionally receive further agents during surgery, such as inhalant anaesthetics, glyceryl trinitrate (GTN), opioid (comprising morphine, fentanyl or remifentanyl). It is extremely relevant to note that these pharmacological agents have been demonstrated to mimic IPC (283) and that therefore it is again possible that RIPC may add no further benefit. Ultimately, in experimental studies no CPB is used, which instead is extensively utilised on patients recruited in the vast majority of RCTs, with the exception of the two studies from Hong et al (284, 285) where only patients undergoing off-pump CABG surgery were enrolled: in the next chapters, this will be extensively explained.

We have so far explained the crucial differences between preclinical and clinical studies and the reasons for the difficult translation of the encouraging outcomes in the former to positive findings in the latter. In addition, other critical differences exist amongst the various published RCTs in this field and this might also in part explain the discrepancies of the results obtained. In the first instance, we have already mentioned that elective cardiac surgery is the clinical setting observed in the vast majority of these trials: intriguingly, one of the so far largest proof-of-concept trials enrolled both stable and unstable patients (286). Importantly, unstable angina (UA) is secondary to

transient ischaemia and could therefore act as a preconditioning stimulus before an MI: there is currently clear evidence that patients who have experienced episodes of UA prior to MI have better outcomes than those with no pre-existing symptoms (287, 288), who conversely represent the closest translation of animal models. A consequence of this is that the additional benefit provided by RIPC in subjects already "preconditioned" by recent angina episodes might only lead to non-significant differences in PMI and clinical outcomes. Furthermore, from table 1.10 it is clear that diabetes has played a major role in the selection of patients recruited into the different clinical studies and this is due to the interference of this condition with the cardioprotective mechanisms of RIPC: in the literature, we have therefore observed trials excluding patients with DM or involving both diabetic and non-diabetic subjects or crucially enrolling diabetic patients only. This important aspect will represent the focus of our attention in chapter 6 where we will describe a retrospective analysis of our principal study involving subgroups with or without DM. Other crucial elements of differentiation exist amongst the clinical studies and consist of:

- the type of operation, including CABG surgery alone, valve surgery alone or a combination of the two, with subsequent different magnitude of PMI sustained;
- the technique of myocardial preservation utilised, comprising ICCF or cardioplegia, and amongst those studies involving cardioplegia only, the delivery and composition of cardioplegia;
- the anaesthetic regime used, with clinical studies utilising a strict anaesthetic regime or protocols related to the anaesthetists' individual experience.

Ultimately, a further critical aspect needs careful consideration and is given by the diversity of the **protective stimulus** applied: indeed the preconditioning stimulus in different clinical studies varies considerably with respect to number of cycles, timing of

delivery (prior to versus after surgical incision), upper or lower limb utilised for the application, blinding and delivery of the stimulus (in the study by Rahman et al (286), the cuff was kept hidden under a surgical gown in order to ensure blindness in the study protocol). Whilst it is therefore clear that further studies are required in order to characterise the preconditioning stimulus in this regard, it is also crucial to emphasise the potentially critical role of the intensity of the preconditioning stimulus, which may not be sufficient to elicit cardioprotection under specific conditions: the majority of the clinical studies have used a standard single-limb RIPC protocol comprising three or four-5 minute cycles of inflation/deflation of a cuff placed on either the upper arm or thigh to induce transient IR. However, several recent studies have failed to demonstrate a significant reduction in PMI using this standard single limb RIPC stimulus, suggesting that this RIPC stimulus may be ineffective in specific settings. Importantly, in our single centre RCT, we have used a simultaneous multi-limb preconditioning stimulus in order to "overcome" potential resistance to the cardioprotective effect.

It is also extremely relevant to highlight fundamental aspects of both positive and negative RCTs: as clearly evidenced in table 1.10, the vast majority of the RCTs investigating the effects of RIPC in the context of cardiac surgery are represented by small proof-of-concept trials, recruiting a limited number of patients in single centers: it is therefore possible that larger multi-centre studies will be able to more accurately evaluate the potential beneficial effects of RIPC. Moreover, most of these trials were single-blinded which may have introduced an element of bias into the final outcomes: intriguingly, it has been suggested that the majority of single-blinded studies were positive, whereas the majority of the double-blinded studies were negative (289). In addition, in most cases, the study primary end-point consisted in the total PMI,

measured by troponin or CK-MB release at specific time-points, either as peak or mean concentration or as a total post-operative AUC: this outcome has been clearly associated with short and long-term morbidity and mortality in these patients (see **table 1.9**), however it represents a “surrogate” endpoint and therefore stronger clinical outcomes are required in order to accurately evaluate the potential effects of RIPC in cardiac surgery. A further significant drawback of the above-mentioned RCTs is patient selection, with exclusion of high-risk patients, particularly those with DM, CKD, recent ACS or undergoing complex cardiac surgery. Crucially in these regards and compared to the so far published proof-of-concept studies, the ERICCA trial has the significant advantages of being a large multi-centre doubled blinded randomised clinical trial with a total of 1612 high-risk patients undergoing CABG surgery with or without valve surgery recruited and with an additive EuroSCORE of at least 5: the study primary study-endpoint is the rate of major cardiac and cerebro-vascular events (MACCE) at 1 year following surgery (290): the results of this trial, available in March 2015, have therefore the potential to change current clinical practice with the application of a simple non-invasive and non-pharmacological intervention.

Table 1.5. Major clinical studies investigating the effects of RIPC in cardiac surgery

Group	Patient group and surgery setting	RIPC Stimulus	Myocardial Injury Outcome	Note
CABG with or without valve surgery				
Hausenloy (282) (2007)	57 Elective CABG Cold-blood cardioplegia and ICCF	Upper-limb ischaemia (3 cycles of 5 min)	↓ AUC of cTnT (43%)	Patients with CKD excluded
Venugopal (291) (2009)	45 Elective CABG±Valve Surgery Cold-blood cardioplegia	Upper-limb ischaemia (3 cycles of 5 min)	↓ AUC of cTnT (42.4%)	Patients with DM and/or CKD excluded
Ali (292) 2010	100 Elective CABG	Upper-limb ischaemia (3 cycles of 5 min)	↓ AUC of CK-MB	Enrolled patients with two or three vessel disease
Thielmann (293) (2010)	53 Elective CABG Cold crystalloid cardioplegia	Upper-limb ischaemia (3 cycles of 5 min)	↓ AUC of cTnI (44.5%)	Diabetic patients excluded
Rahman (286) (2010)	162 Elective CABG Cold-blood cardioplegia	Upper-limb ischaemia (3 cycles of 5 min)	No difference in cTnT, ECG changes, inotrope score, renal and lung injury	Diabetic patients excluded
Karuppasamy (294) 2011	54 Elective CABG Cold-blood cardioplegia and cross-clamp fibrillation	Upper-limb ischaemia (3 cycles of 5 min)	No difference in cTnI, BNP, CK-MB, cytokines or growth factors	All patients received isoflurane before CPB and propofol post-CPB.
Hong (284) 2010	130 Elective off-pump CABG	Upper-limb ischaemia (4 cycles of 5 min)	No significant difference in AUC of cTnI	No RIPC alone or RIPost alone groups.
Wagner (295) 2010	101 Elective CABG Cold crystalloid cardioplegia	Upper limb ischaemia (3 cycles of 5 min)	↓TnI at 8 hours only Tramadol group had ↑ TnI at 8, 16 and 24 hours.	RIPC protocol delivered 18 hours before operation Third group included patients receiving tramadol day before and on the day of the operation
Young (296) 2012	96 High risk cardiac surgery Cold blood cardioplegia	Upper-limb ischemia (3 cycles of 5 min)	↑ hsTnT release in RIPC group.	High risk = CABG + valve surgery, CABG with LVEF<50%, any "redo" operation, MV surgery, double or triple valve surgery
Lomirotov (297) 2012	80 Elective CABG Cold crystalloid cardioplegia	Upper-limb ischemia (3 cycles of 5 min)	No difference in TnI or CK-MB release	Patients with DM and/or CKD excluded TnI and CK-MB measured pre-operatively and at 6, 24 and 48 hours post-CPB but not at 12 and 72 hours post-CPB.
Kottenberg (298) 2012	72 Elective CABG Cold crystalloid cardioplegia	Upper-limb ischemia (3 cycles of 5 min)	Reduction of cTnI AUC only when RIPC given with isoflurane and not propofol	Diabetic patients excluded

Hong (285) 2012	70 Elective off-pump CABG	Lower-limb ischaemia (4 cycles of 5 min)	↓ AUC of cTnI	RIPC was given with RIPost
Lucchinetti (299) 2012	55 Elective CABG Cold blood cardioplegia	Lower limb ischaemia (4 cycles of 5 min)	No difference in TnT, BNP, CRP, S100 protein or long term-clinical outcomes	BP inflated to 300mmHg Anaesthesia induction: opioids, propofol Anaesthesia maintenance: isoflurane
Thielmann (300) 2013	329 Elective CABG Cold crystalloid cardioplegia	Upper-limb ischaemia (3 cycles of 5 min)	↓ AUC of cTnI ↓ All-cause mortality	Diabetic patients excluded
Valve surgery				
Li (301) 2001	40 MVR, AVR, DVR Crystalloid cardioplegia	Aortic cross-clamping (two cycles of 3 minutes of ischaemia and 2 minutes of reperfusion)	Improved pulmonary function and decreased inflammatory response	
Li (302) 2010	81 Elective valve replacement Cold crystalloid-blood cardioplegia	RIPC group: lower limb ischemia (3 x 4 min) before aortic cross-clamping RIPerC group: lower limb ischemia (3 x 4 min) after aortic cross-clamping	RIPC group: no difference in TnI AUC RIPerC group: 40% reduction of peak TnI but no difference in TnI AUC	Preconditioning stimulus: blood pressure inflation around the upper thigh to 600 mmHg Excluded patients with DM, CAD, IE, HTN, PAD affecting lower limbs, and previous cardiac surgery.
Choi (303) 2011	76 Complex valve surgery Blood cardioplegia	Lower limb ischemia (3 x 10 min)	Significant CK-MB reduction at 24 hours only No difference in renal injury biomarkers	CK-MB measured pre-operatively and at 12 and 24 hours post-operatively Complex valve surgery = double-valve surgery, combined valve and CABG procedures, Bentall operation, combined MV and TV annuloplasty or reoperation
Xie (304) 2011	73 Elective valve replacement Cold blood cardioplegia	Upper-limb ischemia (3 x 5 min)	44% reduction in TnI AUC	
Young (296) 2012	96 High risk cardiac surgery Blood cardioplegia	Upper-limb ischemia (3 x 5 min)	Increased hsTnT release in RIPC group.	High risk = CABG + valve surgery, CABG with LVEF<50%, any “redo” operation, MV surgery, double or triple valve surgery
Kim (305) 2012	54 Complex valve surgery Blood cardioplegia	RIPCpre plus RIPost stimulus: 3 x 10 minutes cycles of lower limb ischaemia 10 minutes after anaesthetic induction and prior to CPB discontinuation	No myocardial injury outcome No difference of pulmonary function between intervention groups.	Cuff inflated to 250 mmHg
Wu (306) 2011	75 MVR Blood cardioplegia	LIPC-I (3x5 cycles of upper arm ischaemia) LIPC-II (3x5 cycles of upper arm ischaemia) plus 2x10 min cycles of upper leg ischaemia)	Reduced TnI release in LIPC-II group only	

Corrective paediatric surgery				
Cheung (281) (2006)	37 Elective paediatric cardiac surgery	Lower-limb ischaemia (4 cycles of 5 min)	↓ cTnI ↓ inotrope score ↓ airway resistance	
Zhou (307) 2010	60 Elective surgical repair of simple congenital heart defect	Upper-limb ischaemia (3 cycles of 5 min)	↓ cTnI/CK, CK-MB ↓ systemic inflammatory response ↓ airway resistance	RIPC applied 24 hrs and 1 hr prior to surgery
Pavione (308) 2012	22 Elective paediatric cardiac surgery	Lower-limb ischemia (4 cycles of 5 min)	No significant TnI reduction Significant reduction of NT-proBNP AUC No difference in post-operative inflammatory response	RIPC stimulus given 24 hrs prior to the operation
Jones (309) 2013	39 Elective paediatric surgery	Lower-limb ischemia (4 cycles of 5 min)	No significant difference in PMI, inotropic requirement, renal or cerebral injury	Neonates with transposition of the great arteries or hypoplastic left heart syndrome
McCrindle (310) 2014	299 Elective paediatric cardiac surgey	Lower-limb ischemia (4 cycles of 5 min)	No difference in hospital stay duration	

RIPC=remote ischemic preconditioning; cTnI=cardiac troponin-I; CK=creatine kinase; NT-proBNP=N-terminal pro-brain natriuretic peptide; AUC=area-under-the-curve; CABG=coronary artery bypass graft; cTnT=cardiac troponin-T RIPost=remote ischaemic postconditioning; CKD=chronic kidney disease; DM=diabetes mellitus; LVEF=left ventricular ejection fraction; CPB=cardiopulmonary bypass; BNP=brain natriuretic peptide; CRP=C-reactive protein; MV=mitral valve. DVR=aortic and mitral valve replacement; MVR=mitral valve replacement; LIPC=limb ischaemic preconditioning

1.7.2.2. RIPC and Non-Cardiac Surgery

The setting of major vascular surgery and particularly elective abdominal aortic aneurysm (AAA) repair has been another important field to which the concept of cardioprotection induced by RIPC has been applied (**Table 1.6**): MI was the most common cause of both early and late mortality after elective AAA repair and affected 3.1% of patients in a survey involving 557 subjects (311). Haggart and colleagues (312) demonstrated that cTnI can rise in up 58% of emergency AAA repair operations and 29% of elective cases. Moreover, Barbagallo et al (313) showed that even small early increases of cardiac enzymes can affect morbidity and mortality in patients undergoing major vascular surgery. Crucially, preoperative coronary revascularisation

has not been demonstrated to provide additional benefits on clinical outcome post-AAA repair (314), and this could be as a result of non-haemodynamically significant stenosis (315).

Another important aspect of post-operative complication of major vascular surgery is AKI, which can occur in up to 10% of these patients as a consequence of hypoperfusion secondary to aortic cross-clamp and IRI (316). It also affects morbidity and mortality after elective AAA repair (317) and is an independent predictor of death in these patients (318). It is therefore clear that strategies are required in order to protect the myocardium and the kidney during the perioperative period in these high-risk patients and RIPC offers the potential to provide multi-system protection from PMI, AKI and other major organ injury.

Ali et al (292) were the first group to apply the concept of RIPC to patients undergoing AAA repair: in a study involving 82 subjects, RIPC was given with two cycles of intermittent cross-clamping of the common iliac artery with 10 minutes of ischaemia followed by 10 minutes of reperfusion, and was associated with a reduced incidence of PMI, post-operative MI and AKI. Subsequently, Walsh and colleagues (319) evaluated cardiac and renal injury in 40 patients undergoing endovascular AAA repair (EVAR) using sequential lower limb ischaemia and concluded that there was no difference between preconditioned and control patients with respect to cardiac outcomes, however RIPC subjects had a lower increase in postoperative urinary retinol binding protein (RBP) levels and a lower median urinary albumin/creatinine ratio. In two additional studies (320, 321), the same authors failed to provide beneficial effects of RIPC on cardiac or renal outcomes in the setting of elective open infra-renal aortic aneurysm repair (320), or of elective carotid endarterectomy (CEA) (321), where the preconditioning stimulus applied with 10-minute inflation-deflation of a blood

pressure cuff around both upper thighs sequentially. Finally, Li et al (322) showed that standard upper limb IR reduced pulmonary and intestinal injury in patients undergoing elective open AAA repair, however no benefit was found with RIPC in cardiac or neurological outcomes or hospital/ICU stay.

It is also relevant to note that the on-going Preconditioning Shields Against Vascular Events in Surgery (SAVES) RCT is being conducted in order to determine whether RIPC with 4 cycles of upper arm IR improves MACCE rate at 30 days in patients undergoing aortic aneurysm repair, carotid endarterectomy, lower limb surgical revascularisation and major lower limb amputation for end-stage vascular disease (ClinicalTrials.gov identifier: NCT01691911)

Table 1.6. Major clinical studies investigating the effects of RIPC in vascular surgery

Group	Patient group and surgery setting	RIPC Stimulus	Myocardial Injury Outcome
Ali (292) 2007	41 Elective AAA repair	2 cycles of sequential cross clamping of right and left iliac vessel for 10 min	↓ PMI and AKI
Walsh (319) 2009	18 Elective EVAR	2 cycles of lower limb ischemia an reperfusion (each 10 min)	No difference in cardiac or renal outcomes
Walsh (320) 2009	22 Elective open AAA repair	2 cycles of sequential cross clamping of right and left iliac vessel for 10 min	No difference in cardiac, renal or clinical outcomes
Walsh (321) 2010	34 Elective CEA	2 cycles of lower limb ischemia an reperfusion (each 10 min)	No difference in cardiac or renal outcomes or saccadic latency
Li (322) 2013	31 Elective open infrarenal AAA repair	Upper-limb ischaemia (3 cycles of 5 min)	↓ Pulmonary and intestinal injury and systemic inflammatory response. No difference in renal, neurological or cardiac outcomes

RIPC=remote ischemic preconditioning; AAA=abdominal aortic aneurysm; EVAR=endovascular aortic aneurysm repair; CEA=carotid endo-arterectomy; PMI=peri-operative myocardial injury; AKI=acute kidney injury.

1.7.2.3. RIPC and elective PCI

Patients undergoing elective PCI are subject to PMI in up to one third of cases (323) and, similarly to the context of cardiac and non-cardiac surgery, post-procedure elevation of cardiac biomarkers including CK-MB, TnI, TnT has been associated with worse short and long-term clinical outcomes (324-329). A number of factors have been identified for their significant impact on PMI magnitude in elective PCI and are related to (330):

1. *patient*, such as advanced age (331), systemic atherosclerosis (332), diffuse CAD (332), multi-vessel CAD (332), CKD (333), anaemia (334), CRP elevation (335) and white blood cell count>9.5 $x10^6$ prior to PCI (336);
2. *angiographic lesion*, including calcification, anatomical complexity of the lesion, plaque burden, bifurcation lesions and tortuosity (13, 337);
3. *procedure*, comprising stent length, suboptimal stent insertion, directional coronary atherectomy versus angioplasty.

In the context of PCI, PMI can be caused by two different mechanisms:

a. side branch occlusion (19% of cases) during balloon inflation or stent insertion: it occurs adjacent to the treated coronary segment due to plaque shift, dissection, spasm, embolization and thrombus formation (proximal type or type 1) (338);
b. structural and functional micro-vascular obstruction in the territory distal to the treated segment (50-75% of cases), due to distal embolisation, microvascular plugging secondary to platelet and neutrophils activation, oxidative stress, inflammation, vascular neuro-hormonal modulation (distal type or type 2) (338).

The concept of PMI in the context of elective PCI and the subsequent prognostic value of cardiac biomarkers have therefore led to the application of RIPC to this clinical

setting with the aim to reduce post-procedure myocardial damage and therefore improve patients' clinical outcomes (**Table 1.7)**.

In a first study conducted by Iliodromitis et al (339) on patients with single-vessel CAD undergoing elective uncomplicated PCI, RIPC, given with three-5 minutes cycles of both upper limbs IR, did not attenuate the inflammatory response and was associated with a significant CK-MB and troponin rise. However, it is possible that the latter could be due to the induction of bilateral upper limb ischaemia causing a more severe enzyme leakage in preconditioned patients: moreover, crucially in the clinical setting of elective PCI, minimal PMI is sustained and therefore beneficial effects of RIPC are less evident.

Hoole and colleagues extensively investigated the effects of RIPC in the context of elective PCI (340-343): they first found that RIPC with standard upper limb IR was associated with significantly lower median cTnI at 24 hours after PCI, and reduced incidence of chest discomfort, ST-segment deviation and MACCE rate at 6 months (340) and 6 years (343). However, the same stimulus did not result in reduction of micro-vascular resistance or improvement of coronary flow velocity in patients with single vessel disease or when it was applied through target vessel balloon occlusion (cardiac RIPC) to those with MVD (341). Similarly, RIPC did not improve ischaemic LV dysfunction in patients with single vessel disease (342).

Interestingly, subsequent studies demonstrated that three- (344) or even one-5 minutes (345) cycles of arm RIPC reduced PMI in patients with stable angina undergoing elective PCI however diabetic subjects were not showed to be protected by standard RIPC stimulus (346). Liu and colleagues (347) found that a late RIPC stimulus, applied 18-24 hours prior to elective PCI, reduced incidence of chest pain and ST elevation, and decreased median TnI, CK and CK-MB, although the same

stimulus, applied immediately before elective PCI, was not effective (348, 349). Importantly, a recent meta-analysis confirmed the cardioprotective effects of RIPC in this clinical setting (350) and currently studies are being undertaken in order to evaluate the effects of RIPC on contrast-induced nephropathy (CIN), PMI and clinical outcomes in subjects undergoing elective PCI (351) and on CIN in the context of elective coronary angiography (352).

1.7.2.4. RIPC and Primary PCI

In patients presenting with STEMI, early restoration of blood flow with PPCI is currently the best strategy to reduce infarct size and improve patient morbidity and mortality. Experimental studies have demonstrated that following an AMI, IRI can be responsible for up to 50% of the final infarct size (96): similarly, in humans PMI secondary to IRI might in part explain the high incidence of death and heart failure following AMI (96), which remain as high as 10% (353) and 25% (354) respectively at 1 year. Therefore adjuvant treatments have been investigated to potentiate innate cytoprotective mechanisms and therefore to potentially limit infarct size, preserve LV function and improve clinical outcomes. Crucially the more recent application of RIPC to patients presenting with STEMI and undergoing PPCI represents the closest translation of experimental studies into the clinical scenario (96): the complete occlusion (TIMI-0 flow) of coronary arteries in STEMI patients resembles the direct ligature of the coronary artery of animal models of MI and often these subjects have no pre-existing condition and are on no concomitant medication as it occurs in experimental studies (234).

Table 1.7. Major clinical studies investigating the effects of RIPC in elective or primary PCI

Group	Patient group and clinical setting	RIPC Stimulus	Myocardial Injury Outcome
Iliodromitis (339) 2006	41 Elective PCI	Bilateral ischaemia (3 cycles of 5 min)	↑inflammatory response and PMI
Hoole (340) 2009	202 Elective PCI	Upper-limb ischaemia (3 cycles of 5 min)	↓PMI, MACCE and CP/ST changes
Hoole (341) 2010	54 Elective PCI	Upper-limb ischaemia (3 cycles of 5 min)	No difference in coronary flow velocity or microvascular resistance
Hoole (342) 2010	42 Elective PCI	Upper-limb ischaemia (3 cycles of 5 min)	No difference in myocardial stunning or ventricular dysfunction
Rentoukas (355) 2010	31 Primary PCI	Upper-limb ischaemia (4 cycles of 4 min, 20mmHg>SBP)+tramadol	↓PMI and improved ST-segment elevation resolution
Bøtker (356, 357) 2010	333 Primary PCI	Upper-limb ischaemia (4 cycles of 5 min)	↑ Myocardial salvage at 1 month. No difference in troponin release or MACCE
Ghaemian (358) 2012	80 Elective PCI	Lower-limb ischaemia (2 cycles of 5 min)	↓troponin at 24 hours ↓CP/ST-deviation during procedure
Davies (343) 2013	192 Elective PCI	Upper-limb ischaemia (3 cycles of 5 min)	↓rate of all-cause mortality, non-fatal MI, CVA, hospital admission fro heart failure at 6 years
Er (357) 2013	50 Elective coronary angiography	Upper-limb ischaemia (4 cycles of 5 min, 50mmHg>SBP)	↓contrast-induced nephropathy
Luo (344) 2013	101 Elective PCI	Upper-limb ischaemia (3 cycles of 5 min)	↓PMI and MI 4a. No difference in renal outcomes
Prasad (349) 2013	95 Elective/urgent PCI	Upper-limb ischaemia (3 cycles of 5 min)	No difference in PMI reduction, inflammatory response or circulating endothelial progenitor cell counts
Ahmed (348) 2013	149 Elective PCI	Upper-limb ischaemia (3 cycles of 5 min)	↓troponin at 16 hours No difference in post procedural MI, CKMB, or CRP levels
Xu (346) 2013	200 Elective PCI in diabetic patients only	Upper-limb ischaemia (3 cycles of 5 min)	No difference on PMI or MI 4a
Crimi (359) 2013	96 Primary PCI	Upper-limb ischaemia (3 cycles of 5 min)	↓CK-MB release Improved myocardial oedema and ST-segment elevation resolution
Zografos (345) 2014	94 Elective PCI	Upper-limb ischaemia (1 cycle of 5 min)	↓troponin at 24 hours/MI 4a
Liu (347) 2014	200 Elective PCI	Upper-limb ischaemia (3 cycles of 5 min) 18 hrs prior to PCI	↓troponin/CK-MB at 24 hours ↓ chest pain/ST-changes
Sloth (360) 2014	333 Primary PCI	Upper-limb ischaemia (4 cycles of 5 min)	↓MACCE rate at 3.7 years median follow-up
White (361) 2014	197 Primary PCI	Upper-limb ischaemia (4 cycles of 5 min)	↓hsTnT release, infarct size and myocardial oedema ↑Myocardial salvage
Prunier (362) 2014	151 Primary PCI	Upper-limb ischaemia (3 cycles of 5 min)+/-IPostC (4x 1 min cycles balloon)	↓CK-MB but no difference between RIPerC and RIPerC+IPotstC groups
Manchurov (363) 2014	48 Primary PCI	Upper-limb ischaemia (4 cycles of 5 min)	Improved endothelial function up to a week
Hausenloy 2014, (ERIC-LYSIS, NCT02197117)	519 Primary PCI	Upper-limb ischaemia (4 cycles of 5 min)	↓troponin/CK-MB

RIPC=remote ischemic preconditioning; PCI=percutaneous intervention; PMI=peri-operative myocardial injury; AKI=acute kidney injury; MACCE=major cardiac and cerebrovascular events; CP=chest pain SBP=systolic blood pressure; MI=myocardial infarction; CK=creatine kinase; hsTnT=high-sensitivity troponin-T; RIPerC=remote ischaemic perconditioning; IPostC=ischaemic postconditioning.

Rentoukas et al. (355) were the first group to apply the concept of remote conditioning to the setting of STEMI (**Table 1.7**): the conditioning stimulus was induced by inflating a blood pressure cuff on the upper limb to 20 mm Hg above systolic blood pressure for 4 minutes, followed by deflation for 4 minutes, a cycle which was repeated three times beginning 10 minutes before the estimated time of the first balloon inflation. A total of 96 patients were randomised to control, remote conditioning alone, or remote conditioning plus morphine. Patients receiving the conditioning stimulus plus morphine presented the highest rate of full ST-segment resolution and reduction of ST-segment deviation score during hospitalization, the lowest peak of cTnI and the highest occurrence of ST-segment deviation resolution. However, the study had no group receiving morphine only and therefore no evaluation of the effects of morphine alone on cardioprotection was carried out.

Bøtker and colleagues (356) demonstrated for the first time the beneficial effects of remote conditioning in a large seminal study including 142 patients with evolving STEMI: the stimulus was applied by intermittent upper limb ischaemia through four-5 minute cycles of arm IR during transport to hospital and prior to PPCI. Myocardial salvage index measured with myocardial nuclear scanning at 30 days after PPCI was significantly improved in conditioned patients, although the incidence of major adverse coronary events (death, reinfarction and heart failure) was similar in the two groups. Subsequently, the same authors (360) demonstrated that a similar conditioning stimulus was also able to reduce MACCE rate for a median follow-up of 3.8 years in

patients with STEMI. In addition, Manchurov et al (363) showed improved endothelial function up to a week post-procedure in a similar clinical setting and more recently, Crimi and colleagues (359) found that a standard conditioning stimulus reduced total CK-MB release and improved T2-weighted oedema volumes and ST-segment elevation resolution in patients undergoing PPCI for occluded LAD. Ultimately our group was able to demonstrate that an increased conditioning stimulus (four-5 minutes cycles of upper arm IR) reduced total hsTnT release and CMR-measured infarct size and myocardial oedema, and improved myocardial salvage in STEMI patients receiving PPCI (361). It is important to note that, particularly based on the beneficial outcomes of Botker's group study and on the high risk profile of patients recruited into our ERICCA, we decided to adopt the same preconditioning stimulus consisting of four-5 minutes cycles of upper arm IR in our multi-centre ERICCA RCT (Chapter 5).

1.7.2.5. Protection of organs other than the heart by RIPC

The discovery that the myocardium could be protected by a preconditioning stimulus given "at a distance" soon led to the evaluation of a potential similar protective effect on organs other than the heart, which typically are subjected to IRI in clinical practice, such as the **kidneys** in the context of cardiac and vascular surgery (364, 365), the **lungs** following cardiac, pulmonary and orthopaedic surgery (366-370), the **brain** subsequently to ischaemic insults associated with CVAs or from IRI secondary to CEA (371, 372), and the **liver** (373-377), **skin** (378-380), **pancreas (381)**, **intestine** (382), and **ovaries** (383-387) in the setting of organ resection and/or transplantation. An overview of experimental and clinical studies is given in **Tables 1.8-1.9,** although it is essential to highlight that the vast majority of human studies were small proof-of-concepts trials and therefore not adequately powered. In this regard, large RCT are currently being conducted to determine whether RIPC confers protection to kidneys

(ERICCA and REPAIR trials, see chapter 3), lungs (ClinicalTrials.gov identifiers: NCT01144585 and NCT01344239), liver (ClinicalTrials.gov identifiers: NCT00796588 and NCT00975702), brain (ClinicalTrials.gov identifiers: NCT01739088, NCT01158508, NCT01515072, NCT01175876, NCT01570231, NCT01321749) and gastro-intestinal system (ClinicalTrials.gov identifier: NCT00975702).

Crucially, the outcomes of these studies will give us the essential answer as to whether RIPC is capable to provide systemic multi-organ protection against acute IRI (388).

Table 1.8. Major experimental studies evaluating the effects of RIPC on organs other than the heart

Experimental study	Experimental setting: model	RIPC protocol	Outcomes
Kidney			
Ates (389) 2002	Rat	One-10 min cycle of hepatic IR	↓BUN and improved histology.
Song (390) 2007	Rat	Three-8 min cycles of small intestine IR	↓Cr, BUN and renal morphologic change.
Lazaris (391) 2009	Rat	One-15 min cycle of aortic clamping/declamping	↓lactate/MDA and renal tissue MDA.
Kadkhodaee (392) 2011	Rat	Four-5 min cycles of IR to hindlimb during renal IR (RIPerC+RIPost).	↓Cr/BUN
Wever (393) 2011	Rat	One-12 min or three-4 min cycles of IR to one or both hind limbs. (repeatedx4)	↓AKI except 12 min IR protocol
Wever (394) 2012	Rat	Three-5 min cycles of IR to both hind limbs-RIPost	↓AKI. RIPC effect synergistic with local IPost

Lungs			
Peralta (261) 2001	Rat	One-10 min cycle of hepatic IR	↓inflammatory response
Harkin (263) 2002	Pig	Three-5 min cycles of bilateral hind-limb IR	↓inflammatory response/pulmonary oedema/respiratory failure
Xia (395) 2003	Sheep (myocardial IR)	Three-5 min cycles of iliac artery IR	↓pulmonary vascular resistance/artery pressure
Waldow 2005 (396)	Pig	Three-5 min cycles of CFA IR	↓ALI/pulmonary hypertension
Olguner (262) 2006	Rat (hind-limb IRI)	Three-10 min cycles of hind limb IR	↓inflammatory response
Kharbanda (227) 2006	Pig (myocardial IR)	Four-5 min cycles of hind-limb IR	↑lung compliance, ↓pulmonary resistance
Leung (397) 2014	Mouse (haemorragic stroke/resuscitation)	1 cycle of left femoral artery IR	↓ALT/TNF-α/IL-1 and improved lung histology
Wang (398) 2014	Rat (intestinal IR)	Three-5 min cycles of SMA IR	↓MPO/MDA/TNF-α/IL-1
Liver			
Lai (399) 2006	Rat	Four-10 min cycles of hind-limb IR	↓ALT. ↑HO-1
Kanoria (400) 2006	Rabbit	Three-10 min cycles of hind-limb IR	↓ALT/AST. Preserved PLBF ↑hepatic nitrite/nitrate levels.
Gustaffson (401) 2006	Rat	One-10 min episode of hind-limb IR	↓ALT, no difference in PLBF
Tapuria (402) 2009	Rat	Four-4 min cycles of hind-limb IR	↑hepatic perfusion ↓inflammatory response
Wang (403) 2010	Mouse	One-10 min episode of hind-limb ischemia.	↓ALT/TNF-α ↑HMG-B1.
Abu-Amara (404) 2011	Mouse	Six-4 min cycles of hind-limb IR	↓ALT/AST RIPC blocked by NO inhibitor
Abu-Amara (405) 2011	Mouse	Six-4 min cycles of hind-limb IR	↓ALT/AST
Kanoria (406) 2012	Rabbit	Three-10 min of hind-limb IR	↓ALT/AST ↑mitochondrial oxygenation/hepatic nitrite-nitrate
Abu-Amara (407) 2012	Mouse	Six-4 min cycles of hind-limb IR	↓ALT/AST
Wang (408) 2012	Rat (liver transplantation)	Four-4 min cycles of hind-limb IR	↓ALT/AST/TNF-α.
Uysal (409) 2014	Rat	Three-5 min cycles of hind-limb IR	↓ALT/AST
Wang (410) 2014	Mouse	Six-4 min cycles of hind-limb IR	↓HO1-mediated liver autophagy
Shin (411) 2014	Mouse (hepatic injury in LPS-sepsis)	Three-10 min cycles of hind-limb IR	↓TNF-α/NF-Kb/neutrophil accumulation

Kageyama (412) 2014	Rat	Two-4 minutes cycles SMA clamping/11-minute declamping	↓ALT/AST/TNF-α
Leung (397) 2014	Mouse (haemorragic stroke/resuscitation)	1-cycle of CFA IR	↓ALT/TNF-α/IL-1; improved liver/lung histology
Brain			
Dave (413) 2006	Rat	One-15 or 30 min episode of hind-limbs IR	Preserved CA1-hippocampal neurones
Gurcun (414) 2006	Rabbit (spinal cord IRI)	Two-5 min cycles of 5 min renal IR.	Improved neurological recovery.
Zhao (415) 2007	Rat	Three-10 min cycles of hind-limb IR	↓neurological dysfunction/infarct size
Rehni (416) 2007	Mouse	One-15 min episode of intestinal IR	↓cerebral infarct size/functional deterioration
Ren (417) 2008	Mouse	Three-15 min cycles of hind-limb IR	↓cerebral infarct size
Ren (417) 2009	Mouse	Three-15 min cycles of hind-limb IR (RIPost).	↓cerebral infarct size
Saxena (418) 2009	Rat	Five-5 min cycles of hind-limb IR	No effect on CA1-hippocampal neurones.
Yannopoulos (419) 2010	Pig (CPB)	Four-5 min cycles of hind-limb IR	↓cerebral lactate/glucose/glycerol
Xu (420) 2011	Rat	Three-10 min cycles of hind-limb IR	↑neurocognitive function/Bcl2 expression
Malhotra (421) 2011	Rat	Three-10 min cycles of infra-renal aortic IR	↓cerebral infarct size
Jensen (422) 2011	Pig (CPB)	Four-5 min cycles to hind-limb IR	↓cerebral lactate/histological injury
Zhou (423) 2011	Rat	Four-10 min cycles of hind-limbs IR (RIPost)	↓cerebral infarct size
Hahn (424) 2011	Rat	Four-5 min cycles to hind-limb IR	↓cerebral infarct
Sun (425) 2012	Rat	Three-5 min cycles of hind-limbs IR (RIPost)	↓cerebral infarct size
Geng (426) 2012	Rat	Repeated episodes of hind-limbs IR	No beneficial effects
Wei (427) 2012	Rat	Three-15 min cycles of hind-limb IR.	↓cerebral infarct size/oedema/brain-barrier permeability.
Hu (428) 2012	Rat	Three-5 min cycles of hind-limb IR	↓cerebral infarct size
Yannopoulos (429) 2012	Pig	Four-5 min cycles of hind-limb IR	↑cerebral oxygen tension
Hu (430) 2013	Rat	Four-5 min cycles of hind-limb IR	↑neurofunction
Yannopoulos (431) 2013	Pig	Four-5 min cycles of hind-limb IR	↑mitochondrial respiratory function and ↓inflammatory response

Sichuan 2014	Rat	Lower hind limb IR	↓neurological deficit/infarct size
Hu (432) 2014	Rat (haemorrragic stroke)	Four-5 min cycles of hind-limb IR	↑myocardial indices ↓neurological deficit

IRI=ischaemia-reperfusion injury; RIPC=remote ischaemic preconditioning; RIPost=remote ischaemic postconditioning; RIPerC=remote ischaemic perconditioning; BUN=blood urea nitrogen; MDA= malonedialadehyde; IR=ischaemia reperfusion; ALT=aspartate transaminase; ALT=alanine transaminase; LPD=lipopolysaccharide; TNF-α= tumor necrosis factor-α; MPO= myeloperoxidase; SMA-superior mesenteric artery; TNF=tumor necrosis factor; IL=interleukin; CFA=common femoral artery

Table 1.10. Major clinical studies investigating the effects of RIPC on renal and pulmonary protection in patients undergoing cardiac or vascular surgery or elective PCI

Clinical study	Clinical setting	RIC protocol	Result of RIC
Renal Protection			
Ali (292) 2007	82 Elective AAA repair	10 min right-CIA clamping+ left-CIA clamping	↓renal impairment incidence
Walsh (319) 2009	40 Elective EVAR	10min right thigh inflation+10 min left thigh inflation	↓urinary retinol binding protein
Walsh(433) 2010	40 Elective open infrarenal-AAA repair	10 min right-CIA clamping+ left-CIA clamping	No effect on urinary retinol binding/albumin:Cr ratio
Venugopal (434) 2009	78 Adult CABG surgery	Three-5 min arm IR	↓incidence of AKI
Choi (303) 2011	76 Adult complex valve surgery	Three-10 min thigh IR	No effect on AKI
Zimmerman(435) 2011	120 Adult CABG surgery	Three-5 min thigh IR	↓incidence of AKI
Pedersen(436) 2011	103 Paediatric cardiac surgery	Four-5 min thigh IR	No effect on AKI
Whittaker (437) 2012	43 Adult patients with mild CKD undergoing PPCI	>4 inflations/deflations of angioplasty balloon	Preserved renal function
Er (357) 2012	100 Adult patients with moderate CKD undergoing elective PCI	Four-5 min upper arm IR	↓ contrast-AKI incidence

Pulmonary Protection			
Cheung (281) 2006	37 Paediatric cardiac surgery	Four-5 min cycles of thigh IR	↓airway resistance
Li (301) 2010	40 Adult CABG surgery	Two-3 min cycles aortic cross clamping	↓ventilation requirements/pulmonary oedema/inflammatory response
Zhou (307) 2013	60 Paediatric cardiac surgery	Three-5 min cycles of arm IR	↑lung static+dynamic compliance.
Li (302) 2010	81 Adult valve surgery	Three-4 min cycles of thigh IR	No effect on ventilation time.
Lin (438) 2012	30 Lower limb orthopaedic surgery	Three-5 min cycles of leg IR	↓pulmonary injury.
Young (296) 2011	96 Adult high-risk CABG surgery	Three-5 min cycles of arm IR	↑ventilation time
Kim (439)	54 Adults complex valve surgery	Three-10 min cycles of thigh IR	No effect on PaO2/FiO2/ALI incidence
Lomivorotov (297) 2012	80 Adult CABG surgery	Three-5 min cycles of arm IR	No effect on ventilation time
Renal and Pulmonary protection			
Thielmann (293) 2010	53 Adult CABG surgery	Three-5 min upper arm IR	No difference in Cr/eGFR
Rahman (286) 2010	163 Adult CABG surgery	Three-5 min upper arm IR	No difference in AKI/ventilation time
Hong (285) 2010	70 Adult off-pump CABG surgery	Four-5 min cycles of thigh IR	No difference in Cr/PaO2/FiO2 ratio
Thielmann (300) 2013	329 Elective CABG	Three-5 min upper arm IR	No difference in AKI/ventilation time

AAA=abdominal aortic aneurysm; Cr=creatinine; CABG=coronary artery bypass graft; PCI=percutaneous intervention; AKI=acute kidney injury; RIPC=remote ischaemic preconditioning; PaO_2 =oxygen tension; FiO_2=Fraction of inspired Oxygen ratio; CIA= common iliac artery

1.8. Conclusions

RIPC, in which the application of one or more brief cycles of non-lethal IR to an organ or tissue protects the heart against a lethal episode of acute IRI, has emerged as a non-invasive, low-cost therapeutic intervention for potentially reducing the extent of PMI in patients undergoing CABG and/or valve surgery and elective or primary PCI. It has also been demonstrated to reduce IRI in organs and tissue other than the myocardium, such as the kidneys, lung, liver, brain, skin, gastro-intestinal system and ovaries. With particular regards to the effects of RIPC on PMI in the context of cardiac surgery, the majority of the clinical studies investigating the effects of a preconditioning stimulus on myocardial damage have reported beneficial effects using a standard single-limb RIPC protocol comprising three or four-5 minute cycles of inflation and deflation of a cuff placed on either the upper arm or thigh to induce transient limb ischaemia. However, several recent studies have failed to demonstrate a statistically significant PMI reduction using this standard single limb RIPC stimulus, suggesting that under certain conditions this RIPC stimulus may be ineffective. This can be attributed to different potential mechanisms including the clinical setting, the patient selection and the intensity and modality of delivery of the preconditioning stimulus. Whether increasing the intensity of the RIPC stimulus by simultaneously applying the RIPC protocol to the upper arm and thigh may be more effective in inducing PMI reduction and improving short-term clinical outcomes in patients undergoing CABG and/or valve surgery is unknown and its implications will be extensively described in the next chapters.

CHAPTER 2

2. Effect of multi-limb remote ischemic preconditioning on clinical outcomes in patients undergoing cardiac bypass surgery

Aims and Objectives

During cardiac surgery, the myocardium is subjected to PMI, as demonstrated by post-operative rise of cardiac enzymes concentrations, including CK-MB (119), TnT (77-79), TnI (80, 81): this has been associated with worse short and long-term clinical outcomes (77-82), with therefore a significant impact on patients' morbidity and mortality.

Acute IRI secondary to intermittent aortic cross-clamp, ICCF or intermittent or continuous administration of cardioplegia (83) has been recognised as one of the crucial mechanisms underlying PMI in this context. RIPC, describing the phenomenon by which brief episodes of transient limb ischaemia to one organ or tissue prior to a prolonged period of ischaemia in a distant or "remote" organ or tissue protect this organ/tissue from IRI, offers a promising non invasive and risk-free strategy to reduce PMI in these subjects and therefore to potentially improve their short and long-term prognosis (96).

A significant number of RCTs have been carried out in order to determine the cardioprotective effects of RIPC in the context cardiac surgery, albeit with often discordant outcomes **(Tables 1.6)**: we discussed the potential reasons for these findings in chapter 1 and identified in the intensity of the preconditioning stimulus one of the possible causes of the failure to significantly reduce PMI in some of the RCTs.

2.1. Hypothesis

We hypothesised that an enhanced RIPC stimulus with two-5 minutes cycles of simultaneous IR to the upper arm and upper thigh reduces PMI and improves short-term clinical outcomes in patients undergoing CABG and/or valve surgery.

2.2. Overall Aim

To investigate the effects of an enhanced RIPC stimulus on PMI and short-term clinical outcomes in patients undergoing cardiac surgery, including CABG surgery alone, valve surgery alone or a combination of the two.

2.3. Objectives

2.1.3.1. To investigate the effects of multi-limb RIPC on PMI and short-term clinical outcomes in an unselected population including patients undergoing CABG surgery, valve surgery alone or CABG plus valve surgery.

Short-term clinical outcomes investigated in our study include:

1. AKI score in the first 72 post-operative hours.
2. Inotrope requirement during the first 3 post-operative days.
3. Length of ICU stay.
4. Length of hospital stay.
5. New onset AF in the first 72 hours after surgery.
6. Adverse events during hospital stay including:
    - Skeletal muscle injury.
    - Death.
    - Non-fatal MI.
    - Coronary artery revascularization.
    - Stroke.
7. Clinical outcomes at 6 weeks:
    - Death.
    - Non-fatal MI.
    - Coronary artery revascularization.
    - Stroke.

2.1.3.2. To investigate the effects of multi-limb RIPC on PMI and short-term clinical outcomes in patients undergoing:

a. CABG surgery, with or without valve surgery, using cardioplegia as the only technique of myocardial preservation;
b. CABG surgery, with or without valve surgery, using ICCF as the only technique of myocardial preservation;

c. CABG surgery with or without valve surgery;
d. CABG surgery alone;
e. CABG surgery alone using cardioplegia as the only technique of myocardial preservation;
f. valve surgery alone, including AVR alone or mitral valve (MV) surgery (replacement or repair);
g. cardiac surgery, with or without intra-operative administration of intra-intravenous (iv) nitrates.

2.1.3.3. To investigate the effects of multi-limb RIPC on PMI and short-term clinical outcomes in diabetic and non-diabetic patients undergoing:

a. cardiac surgery, including CABG surgery, valve surgery alone or CABG plus valve surgery;
b. CABG surgery, with or without valve surgery, using cardioplegia as the only technique of myocardial preservation;
c. CABG surgery alone;
d. CABG surgery alone using cardioplegia only as the only technique of myocardial preservation.

2.1.3.4. To investigate the effects of combined antegrade and retrograde cardioplegia compared to antegrade cardioplegia alone and ICCF alone on PMI and short-term clinical outcomes in control patients undergoing CABG surgery.

CHAPTER 3

3. Effect of multi-limb remote ischemic preconditioning on clinical outcomes in patients undergoing cardiac bypass surgery

Methods and Results

3.1. Overview

We conducted a single-centre single-blinded randomised controlled clinical trial between December 2010 and July 2012 in order to investigate the effects of an enhanced RIPC stimulus induced by transient simultaneous multi-limb IR on PMI and short-term clinical outcomes in an unselected population of adult patients undergoing CABG surgery or valve surgery or a combination of the two. A schematic overview of the study design is given in **Fig. 3.1**.

3.2. Ethical approval and informed consent

This parallel single-blinded randomised controlled clinical trial received local Ethics Committee approval, and was conducted at the Heart Hospital, University College London Hospital (London, UK), in accordance with the International Conference of Harmonisation-Good Clinical Practice (ICH-GCP) guidance. Study protocol, patient information sheet and consent forms were approved by the joint University College

London and University College London Hospital NHS Trust Committees for the ethics of human research. I provided major and minor amendments to previous study protocols, which received approval from the local ethical committee. Eligible patients were approached the day before or on the day of their admission and prior to their surgery, so that they had sufficient time to provide their informed consent when agreed. Written consent was obtained from all patients recruited into the study. I then obtained two photocopies of the signed consent form: the original copy was added to the patient's medical notes, one copy was given to the patient and one copy was kept in a separate file at the Hatter Cardiovascular Institute.

3.3. Study Design

3.3.1. Original Hypothesis and Experimental Design

The study was originally designed in 2001 in order to establish whether the effects of IPC in diabetic patients undergoing cardiac surgery might be abrogated by glibenclamide (**substudy 1**) and was stopped as this anti-diabetic medication was used very rarely. It was then amended in 2006 (**substudy 2**) to establish whether RIPC protects non-diabetic or diabetic patients undergoing cardiac surgery.

As already mentioned in chapter 1, recent RCTs investigating the effects of RIPC on PMI and clinical outcomes in patients undergoing cardiac surgery have provided discrepant results. More recently, an increased intensity of the RIPC stimulus has been found to be beneficial in patients presenting with STEMI and undergoing PPCI and, crucially, preclinical studies have demonstrated that the diabetic myocardium requires a higher threshold for cardioprotection to be achieved prior to a prolonged period of ischaemia (440).

I therefore provided the amendments to previous study protocol and obtained ethical approval, in order to investigate whether:

- an enhanced preconditioning stimulus protects patients undergoing cardiac surgery (**substudy 3)**;
- an enhanced preconditioning stimulus protects both diabetic (**substudy 3a)** and non-diabetic (**substudy 3b)** patients undergoing cardiac surgery.

The enhanced preconditioning stimulus for which I obtained ethical approval consisted of 2 cycles of simultaneous blood pressure (BP) cuff inflations, one placed around the upper arm and one placed around the upper leg for 5 minutes followed by deflation for 5 minutes.

3.4. Patient recruitment

Adult patients were screened against inclusion and exclusion criteria and recruited if considered eligible to the study and following informed consent between December 2010 and July 2012.

3.4.1. Inclusion criteria

a. Age more than 18 years.
b. Patients undergoing CABG surgery and/or valve surgery at the Heart Hospital (UCLH).
c. Patient given informed consent.

3.4.2. Exclusion criteria

a. Cardiogenic shock or cardiac arrest preceding surgery.

Cardiogenic shock was defined as:

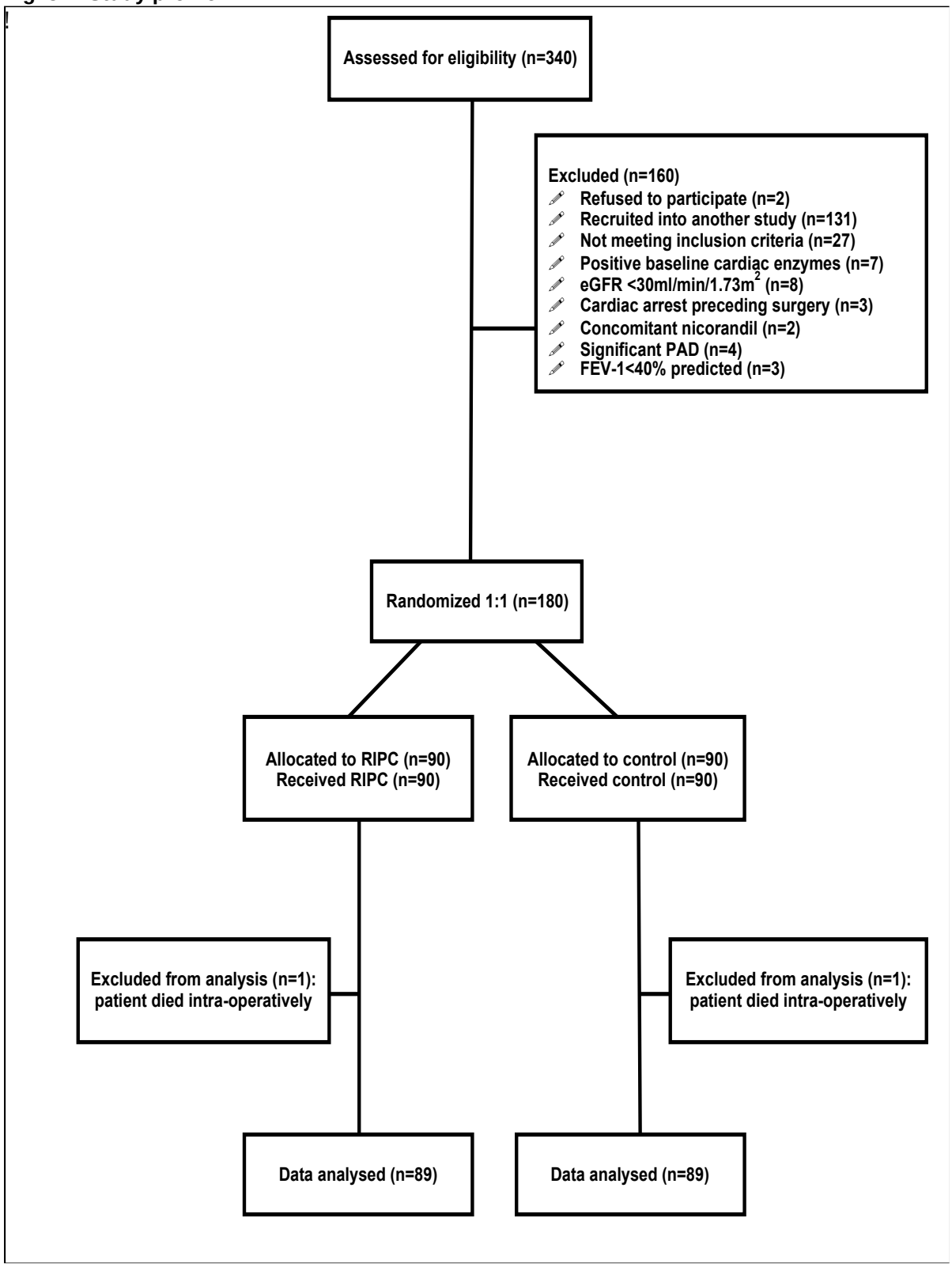
- Systolic BP (SBP)<90 mmHg for 30 minutes before inotrope/vasopressor administration

  or

- Vasopressors or intra-aortic balloon pump (IABP) required to maintain SBP>90 mm Hg.

b. Positive baseline serum hsTnT and/or CK-MB. We excluded these patients, as it was possible that they might have been already preconditioned by the recent event, with a subsequent potential impact on RIPC prior to cardiac surgery.
c. Pregnancy.
d. Significant peripheral arterial disease (PAD) affecting upper and/or lower limbs.
e. Significant hepatic dysfunction, with an International Normalized Ratio (INR)>2.0.
f. Significant pulmonary disease, with a Forced Expiratory Volume (FEV)-1<40% predicted.
g. Renal failure with an estimated glomerular filtration rate (eGFR)<30mL/min/1.73 m^2. These patients were excluded as the excretion of troponin occurs via the kidneys and is therefore impaired in the presence of acute or chronic kidney disease thereby making hsTnT evaluation unreliable.
h. Concomitant therapy with glibenclamide or nicorandil, as these medications may interfere with RIPC (171, 441).

Fig. 3.1. Study profile



RIPC=remote ischaemic preconditioning; eGFR=estimated glomerular filtration rate; PAD=peripheral arterial disease; FEV=forced expiratory volume

3.5. Randomisation

Randomisation was carried out using a computer-generated list of randomised numbers, and allocation concealment obtained using Sequentially Numbered Opaque Sealed Envelopes (SNOSE). In all patients it occurred prior to transfer to the anaesthetic room on the day of surgery.

3.6. Blinding

This was a single-centre, single blinded randomised control clinical trial. The investigator collecting and analysing the data, patients, cardiac surgeons and anaesthetists, operating theatre staff, and staff on ICU and cardiac wards were all blinded to treatment allocation.

3.7. Intervention: RIPC and sham treatment protocols

RIPC and control protocols were applied after anaesthesia induction and prior to sternotomy. RIPC was delivered with one standard BP cuff placed on the upper arm and another standard BP cuff placed on the upper thigh. The cuffs were simultaneously inflated to 200 mmHg and left inflated for 5 minutes, to be then deflated to 0 mmHg and left deflated for 5 minutes. This cycle was repeated twice, so that the total duration of the intervention was 15 minutes. If the patient's SBP was greater than 185 mmHg, the cuffs were inflated to 15 mmHg above that level.

For the control protocol, the two cuffs were simultaneously placed on the upper arm and the upper thigh and left un-inflated for 15 minutes.

The arm BP cuff was placed contralaterally to the arm used for arterial line insertion and invasive BP monitoring. When possible, the leg BP cuff was placed ipsilaterally to the arm cuff in order to facilitate the operator's delivery of the intervention. Both RIPC and sham protocols were delivered following induction of anaesthesia to avoid patient's discomfort.

3.8. Anaesthetic procedure

Patients received pre-medication with oral temazepam 10-20 mg one hour prior to surgery. The patient was then taken to the anaesthetic room where iv access was gained through a peripheral venous cannula and continuous invasive BP monitoring was achieved with the insertion of an arterial line.

Anaesthesia induction was obtained with a combination of midazolam, etomidate, propofol, fentanyl and anti-nicotinic agents including rocuronium, vecuronium or pancuronium. Following anaesthesia induction, the trachea was intubated and mechanical ventilation commenced with oxygen with or without air. Anaesthesia maintenance was achieved with volatile anaesthetic agents, including isoflurane or sevoflurane, and propofol infusion, with or without fentanyl. Arterial BP, central venous pressure, leads I and III of the electrocardiogram and nasopharyngeal temperature were continuously recorded. Intravenous glyceryl trinitrate (GTN) infusion was administered at the discretion of the anaesthetist in order to optimise BP control and improve intraoperative coronary vasodilatation.

3.9. Surgical procedure

Following anaesthesia, the patient was transferred to theatre were mid-line sternotomy was performed: at that point the left IMA (LIMA) was isolated from the thoracic wall if needed and great saphenous vein was harvested as necessary.

Standard non-pulsatile CPB was employed using a membrane oxygenator and cardiotomy suction and further to cannulation of the aortic root and the right atrial appendage: the proximal end of each anastomosis was created during CPB with the distal end to the coronary arteries being constructed during cardiac standstill achieved with aortic root cross-clamp and either induction of ventricular fibrillation or injection of a cardioplegic solution.

With the technique of ICCF, ventricular fibrillation was induced through the application of an alternating current to the epicardium and following aortic root clamping. The distal end of each anastomosis was then constructed, following which the aortic root was declamped and ventricular fibrillation was reverted through a direct current shock.

With regards to the cardioplegic technique of myocardial preservation, this was achieved through two different methods:

1) antegrade cardioplegia: 1 litre of cold blood cardioplegic solution (1 part of St. John's Cardioplegia solution mixed with 4 parts of cold blood) was delivered to myocardial cells through the aortic root further to aortic cross-clamp, followed by a maintenance cold blood cardioplegia, which was given down the grafts in occluded arteries and also into the aortic root every 20-30 minutes. Systemic temperature in these patients was 28-32 ^{0}C;
2) antegrade and retrograde cardioplegia: an initial 800ml dose of antegrade cardioplegia was administered into the aortic root followed by 400ml of

retrograde cardioplegia solution given through the coronary sinus. Following this, maintenance was achieved with 100ml of retrograde cardioplegia after each anastomosis. A hot shot of warm blood without potassium was given after the LIMA anastomosis and prior to removal of the cross-clamp. All anastomoses were constructed with the single-clamp technique. Systemic temperature in these patients was 35 ^{0}C.

With the completion of the anastomosis of the grafts, CPB was discontinued, rewarming was initiated and protamine was used in order to achieve heparin reversal.

3.10. Study Endpoints: rationale and assessment

3.10.1. Study primary end-point: PMI

The study primary end-point was PMI, measured by the area under the curve (AUC) of the total release of hsTnT over the 72 post-operative hours. As previously described, post-operative release of hsTnT may have a significant impact on short and long-term clinical outcomes (77-82) and the potentially insufficient intensity of the preconditioning stimulus may in part explain the negative outcome of recent proof-of-concept studies investigating the effects of RIPC on PMI in patients undergoing cardiac surgery. We therefore intended to establish whether an enhanced RIPC stimulus may reduce PMI in our patient cohort.

Samples were collected pre-operatively and at 6, 12, 24, 48, 72 hours following discontinuation of CPB; hsTnT was measured quantitatively by a one-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche, Switzerland). This assay allows detection of concentrations <1.0 ng/L and measures

the upper range limit (URL) with a coefficient of variation (CV)<10%. The threshold level of ≥14 ng/L indicates significant myocardial necrosis. The unit for reporting was ng/L and the reference range was ≤14 ng/L (14 ng/L is the 99th centile of reference population with CV risk of <10%).

Absolute hsTnT release over the 72 post-operative period was calculated with AUC using the following Excel Office 2010 formulas:

1) AUC between two specific time-points (for example time-points 1 and 2):

$$AUC\ t_1\text{-}t_2 = [(hsTnT \text{ at } t_1 \text{ hours} + hsTnT \text{ at } t_2 \text{ hours})/2] \times (t_2\text{-}t_1)$$

2) Total AUC over the three-post-operative days:

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

3.10.2. Study Secondary Endpoints

3.10.2.1. AKI

In the context of cardiac surgery AKI can occur in up to 30% of patients requiring dialysis in 1-2% of patients and leading to an 8-fold increase in the death (364, 365): RIPC has become a promising non-invasive strategy potentially able to reduce incidence and severity of AKI post-cardiac surgery.

Peri-operative AKI was calculated with the AKI Score over the first 3 post-operative days through a combination of laboratory and clinical data **(Table 2.1.) (442):**

1. Serum Creatinine was measured pre-operatively and at 24, 48, 72 hours following discontinuation of CPB.
2. Urine volumes were monitored daily and calculated as urine output at 24, 48, 72 hours and the total of these individual values.

AKI was therefore classified into three grades derived from Riffle's criteria **(Table 2.1)** (442).

Table 3.1. AKI score as modified from Riffle's criteria (442)

AKI Grade	Creatinine criteria	Urine output criteria
1	Creatinine rise>26.4 µmol/L or 150-200% of baseline	<0.5ml/kg/hr for >6 hours
2	Creatinine rise 200-300% of baseline	<0.5ml/kg/hr for >12 hours
3	Creatinine rise>300% or >354 µmol/L with an acute rise of at least 44µmol/L	<0.3ml/kg/hr for >24 hours or anuria for 12 hours

AKI=acute kidney injury

3.10.2.2. Inotrope requirement

Post-operative inotrope requirement is a crucial reflection of the outcome of cardiac surgery and has in turn the potential of significantly impact on total ICU and hospital stay and ultimately on NHS resources. The inotrope score adapted from a study by Ko and co-workers (443), provides an objective measurement of the requirement of inotropes in the immediate postoperative period: data were collected daily from the medical drug chart on ICU and calculated at 0 (time when CPB was terminated), 24, 48 and 72 hours after CPB discontinuation using the formula provided below.

Inotrope score = *Dosages (in µg/kg/min) of*:

[Dopamine + Dobutamine + Dopeximine]+ [(Adrenaline + Noradrenaline + Isoproterenol) x 100] + [(Enoximone + Milrinone) x 15]

The inotrope score for each time point was calculated as follows: at time 0, the inotrope score was calculated from the dose of the individual inotropes used at the

time of coming off bypass. For 24, 48 and 72 hour time-points, the inotrope score was calculated from the maximum dose of the individual inotropes used in the previous 24 hour period.

3.10.2.3. ICU and hospital stay

The length of ICU and hospital stay is an important post-operative parameter and represents a significant component of NHS costs and resources in patients undergoing cardiac surgery. ICU stay was calculated using ICU chart and medical notes based on numbers of days on ICU and in hospital respectively. When the patient was considered fit for transfer from ICU to the ward although the transfer could not occur for reason not directly related to the patient's condition (i.e. bed not available), ICU stay was counted up to the day when it was clearly documented in the medical notes that it was appropriate for the patient to be transferred from ICU. Similarly, when the patient was considered fit for discharge from hospital but this could not take place due to various reason (social reasons, family reasons), the number of days of hospital stay was again counted up to the day that medical notes clearly documented patient's fitness for hospital discharge.

3.10.2.4. New onset of post-operative AF

New onset of post-operative AF occurs in 30-50% of patients following cardiac surgery (30% post-CABG surgery, 40% following valve surgery and 50% further to CABG plus valve surgery) (444), and is associated with worse morbidity and mortality (444). Post-operative AF is secondary to hypovoloemia, electrolyte imbalance, central venous

catheters insertion, prolonged aortic cross-clamp times, increased automaticity, increased sympathetic tone and importantly acute myocardial IRI (444). Therefore we intended to establish whether an enhanced preconditioning stimulus may protect patients from new post-operative AF in these subjects. This was calculated as the incidence of new onset AF in the first 72 hours after surgery detected by continuous telemetry and ECG (performed by a blinded staff nurse on a daily basis and immediately after the detection of AF on the telemetry, and then analysed by a blinded investigator) and requiring intervention with pharmacological treatment and/or direct current cardioversion. Patients with known permanent AF or paroxysmal AF were excluded from the evaluation of this secondary end-point.

3.10.2.5. Skeletal muscle injury

Skeletal muscle injury was measured to evaluate the magnitude of IRI of upper arm and thigh muscles subsequent to potential damage caused by the increased preconditioning stimulus. It was analysed with CK levels measured pre-operatively and at 6, 12, 24, 48 and 72 hours post-discontinuation of CPB. Total release was calculated as AUC of total CK release over the first 72 post-operative hours, using a similar formula to the one utilised for the evaluation of hsTnT AUC.

3.10.2.6. Short-term clinical outcomes

We calculated the incidence of MACCE in order to assess whether an enhanced preconditioning stimulus may have an impact on short-term clinical outcomes. This

was based on the rate of cardiovascular death, non-fatal MI, coronary artery revascularization, and stroke up to discharge and at six weeks outpatient follow-up.

1. *Cardiovascular death* was defined as death due to a known cardiovascular cause or where the cause of death was unknown i.e. where no other cause of death was identified from the medical history or an autopsy.
2. *Myocardial infarction* included both peri-operative MI and MI following cardiac surgery.
    - Peri-operative MI (type 5 MI) (97) was indicated by hsTnT rise more than five times the 99th percentile of the upper reference limit (URL) during the first 72 hours following surgery, when associated with the appearance of new pathological Q-waves or new LBBB, or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium.
    - Post-surgical MI was defined as a rise and/or fall of hsTnT with at least one value above the 99th percentile of the URL together with evidence of myocardial ischaemia with at least one of the following:
        - Symptoms of ischaemia
        - ECG changes indicative of new ischaemia (new ST-T changes of new LBBB)
        - Development of Q waves in the ECG
        - Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.
        - Sudden unexpected cardiac death involving cardiac arrest often with symptoms suggestive of myocardial ischaemia and accompanied by presumably new ST elevation or new LBBB and/or fresh thrombus on

coronary angiography and/or at autopsy, but death occurring before blood samples could be obtained or at time before the appearance of hsTnT in the blood.

1. *Repeat revascularisation* was defined as any repeat PCI or CABG with or without valve surgery within the first year post-surgery.
2. *Stroke* was defined as a focal, central neurological deficit lasting more than 72 hours and resulting in irreversible brain damage or body impairment.

3.11. Statistical analysis and sample size estimation

Data are presented as mean (standard deviation (SD)) or median (inter-quartile range (IQR)). Comparison between treatment groups was made using unpaired Student T-Test for approximately normally distributed variables or Wilcoxon-Mann-Whitney test for non-normal data. For outcomes collected at different time points a repeated measures linear regression model was used to estimate the difference at each time point and 95% confidence intervals. Categorical data were analysed using Fisher's exact test. The post-hoc analysis of associations between RIPC and GTN was performed using an interaction test in a linear regression model. A value of $P<0.05$ was considered significant. We hypothesised that RIPC would reduce hsTnT AUC by a standardised difference of 0.6. At 90% power and significance at the two-sided 5% level, this required a sample size of 60 subjects, which we increased by 33% to accommodate withdrawal or missing data points. A sample size of at least 80 patients per intervention group was determined based on the following assumptions: (a) the largest study on RIPC in PMI published at the time of this trial initiation (286); (b) a

power of least 90%; (c) a SD of 0.2µg/L; and (d) type I error rate of 5%. Analysis was by intention-to-treat. No adjustment for multiplicity was applied for secondary outcomes or post-hoc analyses. Data were analysed using Stata version 12.1.

3.12. Results: Total unselected cohort of patients undergoing cardiac bypass surgery

A total of 340 patients were assessed for eligibility **(Figure 3.1)**. Of these 160 patients were excluded from the study as 2 patients refused to take part, 131 were included in the ERICCA trial, 27 did not meet inclusion criteria and the remaining subjects had exclusion criteria, with 7 patients having positive cardiac enzymes at the time of potential enrolment, 8 an eGFR<30 ml/min/1.73m^2, 3 presenting with cardiac arrest prior to surgery, 2 being on concomitant nicorandil, 4 having significant PAD and 3 an FEV-1<40% predicted. Therefore 180 patients were recruited and randomised to receive either RIPC (n=90) or control (n=90): 2 patients, 1 in each intervention group, died intra-operatively or in the immediate post-operative period thereby leaving 178 subjects for the final analysis**.** RIPC protocol was completed within 45 minutes of the first aortic cross-clamp in all patients. No significant difference was found between the two treatment groups with respect to baseline patient characteristics **(Table 3.2)**.

The following characteristics had missing values: body mass index (Control 2, RIPC 2), systolic BP (Control 2, RIPC 2), diastolic BP (Control 2, RIPC 2), heart rate (Control 2, RIPC 2), New York Health Association (Control 3, RIPC 4), Canadian Cardiovascular Society (Control 3, RIPC 4), other co-morbidities (Control 1, RIPC 1),

aspirin (Control 2, RIPC 3), clopidogrel/prasugrel (Control 2, RIPC 3), warfarin (Control 2, RIPC 3), beta-blocker (Control 2, RIPC 3), calcium-channel blocker (Control 2, RIPC 3), statin (Control 2, RIPC 3), Angiotensin-Converting-Enzyme-Inhibitor/Angiotensin receptor blocker (Control 2, RIPC 3), long-acting nitrates (Control 2, RIPC 3), insulin (Control 2, RIPC 3), biguanide (Control 2, RIPC 3), sulphonylurea (Control 2, RIPC 3), diuretics (Control 2, RIPC 3).

Similarly operative parameters were similar in the two groups with the only exception of the use of intra-operative GTN, which was significantly higher in the sham group (65 patients vs 53, p=0.035; **Table 3.3.**). In particular, no statistically significant difference was found between the two groups in terms of additive EuroSCORE, CPB and aortic cross-clamp times and use of anaesthetic agents. The following procedure variables had missing values: CPB time (Control 2, RIPC 1), cross-clamp time (Control 4, RIPC 1), anti-nicotinic agents (Control 5, RIPC 4), midazolam (Control 5, RIPC 4), etomidate (Control 3, RIPC 4), fentanyl (Control 3, RIPC 4), propofol on induction (Control 3, RIPC 4), propofol during maintenance (Control 3, RIPC 4), volatile anaesthetics (Control 3, RIPC 4), intra-operative GTN (Control 3, RIPC 1). There were no untoward consequences or side effects with the RIPC protocol.

Table 3.2. Patient baseline characteristics

Patients	Control (n=89) (mean±SD)	RIPC (n=89) (mean±SD)	P value
Age (*years*)	66±10	65±10	0.268
Gender			0.469
Male	67 (75%)	72 (81%)	
Female	22 (25%)	17 (19%)	
Ethnicity			0.649
Caucasian	74 (83%)	71 (80%)	
Asian	10 (11%)	12 (13%)	
Afro-Caribbean	4 (5%)	6 (7%)	
Chinese	1 (1%)	0 (0%)	
BMI			
SBP *(mmHg)*	28.4±5.5	28.8±7.1	0.700
DBP *(mmHg)*	130.0±18.0	129.0±15.7	0.710
HR *(bpm)*	70.7±9.0	70.8±9.4	0.948
	69.2±11.7	66.3±9.8	0.079
Smoking History			0.720
Smoker	12 (14%)	11 (12%)	
Ex-smoker	52 (58%)	48 (54%)	
Non-smoker	25 (28%)	30 (34%)	
Family History of IHD	57 (64%)	64 (72%)	0.335
NYHA Class			0.056
0	8 (9%)	8 (9%)	
I	22 (26%)	31 (36%)	
II	38 (44%)	39 (46%)	
III	17 (20%)	7 (8%)	
IV	1 (1%)	0 (0%)	
CCS Class			0.437
0	30 (35%)	25 (29%)	
I	17 (20%)	19 (22%)	
II	30 (35%)	30 (35%)	
III	7 (8%)	9 (11%)	
IV	2 (2%)	2 (2%)	
LVEF			0.351
>50%	70 (79%)	67 (75%)	
30%-50%	17 (19%)	16 (18%)	
<30%	2 (2%)	6 (7%)	
Co-morbidities			
Diabetes mellitus	24 (27%)	28 (32%)	0.621
Hypertension	70 (79%)	65 (73%)	0.484
Hypercholesterolemia	64 (72%)	68 (76%)	0.608
Atrial Fibrillation	16 (18%)	10 (11%)	0.390
Previous MI	23 (26%)	28 (32%)	0.507
Previous PCI	11 (12%)	11 (12%)	1.000
Previous CVA/TIA	9 (10%)	5 (6%)	0.149
Previous Cardiac Surgery	2 (2%)	4 (5%)	0.600
Other co-morbidities	35 (40%)	32 (36%)	0.427
Peripheral Arterial Disease	6 (7%)	1 (1%)	0.118
Drug History	66 (76%)	72 (84%)	0.390
Aspirin	27 (31%)	24 (28%)	0.239
Clopidogrel/Prasugrel	9 (10%)	6 (7%)	0.710
Warfarin	55 (63%)	57 (66%)	0.543
Beta-blocker	32 (37%)	22 (26%)	0.098
Calcium Channel Blocker	72 (83%)	72 (84%)	0.400
Statin	61 (70%)	57 (66%)	0.494
ACE-I/ARB	14 (16%)	12 (14%)	0.368
Long acting nitrates			
Antidiabetics	7 (8%)	8 (9%)	0.521
Insulin	16 (18%)	19 (22%)	0.724
Biguanide	11 (13%)	7 (8%)	0.611
Sulphonylurea	27 (31%)	31 (36%)	0.600
Diuretics			

SD=standard deviation; RIPC= Remote Ischaemic Preconditioning; NYHA= New York Health Association; CCS= Canadian Cardiovascular Society; MI= Myocardial infarction; PCI= Percutaneous coronary intervention; CVA= Cerebrovascular accident; TIA= Transient ischaemic attack; ACE-I= Angiotensin-converting enzyme inhibitor; ARB= Angiotensin receptor blocker; LVEF= left ventricular ejection fraction.

Table 3.3. Details of surgical procedure

Patients	Control (n=89) (mean±SD)	RIPC (n=89) (mean±SD)	P value
Indication for Surgery			0.661
Angina	44 (49%)	40 (45%)	
Myocardial Infarction	12 (14%)	19 (21%)	
Valve Disease	23 (26%)	23 (26%)	
Angina and Valve Disease	7 (8%)	4 (5%)	
Myocardial Infarction and Valve Disease	1 (1%)	2 (2%)	
Infective Endocarditis	2 (2%)	1 (1%)	
EuroSCORE	3.72±2.03	3.70±2.59	0.949
Additive perioperative risk			0.356
Low (EuroSCORE 0-2)	26 (29%)	29 (33%)	
Medium (EuroSCORE 3-5)	47 (53%)	38 (43%)	
High (EuroSCORE >5)	16 (18%)	22 (25%)	
Bypass-time (min)	96.7±32.6	89.6±31.0	0.145
Cross-clamp time (min)	64.8±26.4	61.5±26.9	0.419
Cardioprotection			
Blood cardioplegia	73 (82%)	75 (84%)	0.297
Cross-clamp fibrillation	16 (18%)	14 (16%)	0.842
Operation			0.994
CABG alone	54 (61%)	57 (64%)	
AVR alone	15 (17%)	14 (16%)	
CABG+AVR	10 (11%)	9 (10%)	
MVR or MV Repair	9 (10%)	8 (9%)	
AVR+MVR	1 (1%)	1 (1%)	
Number of grafts			0.713
One	4 (6%)	5 (8%)	
Two	19 (30%)	15 (23%)	
Three	29 (45%)	35 (53%)	
Four	12 (19%)	11 (17%)	
Anesthetic agents			
Induction			0.274
Anti-nicotinic agents			
Rocuronium	68 (81.0%)	76 (89%)	
Pancuronium	14 (17%)	6 (7%)	
Vecuronium	2 (2%)	3 (4%)	
Midazolam	45 (54%)	33 (39%)	0.265
Etomidate	8 (9%)	7 (8%)	0.805
Fentanyl	86 (100%)	85 (100%)	1.000
Propofol	76 (88%)	77 (91%)	0.637
Maintenance			
Propofol	86 (100%)	85 (100%)	1.000
Volatile Anesthetics			0.527
Isoflurane	80 (93%)	81 (95%)	
Sevoflurane	6 (7%)	4 (5%)	
Intra-operative GTN	65 (76%)	53 (60%)	0.035

SD=standard deviation; RIPC= Remote Ischaemic Preconditioning; CABG= Coronary artery bypass graft; AVR=Aortic valve replacement; MVR= Mitral valve replacement; MV= Mitral valve; GTN=glyceryl trinitrate

3.12.1. RIPC reduced the magnitude of PMI

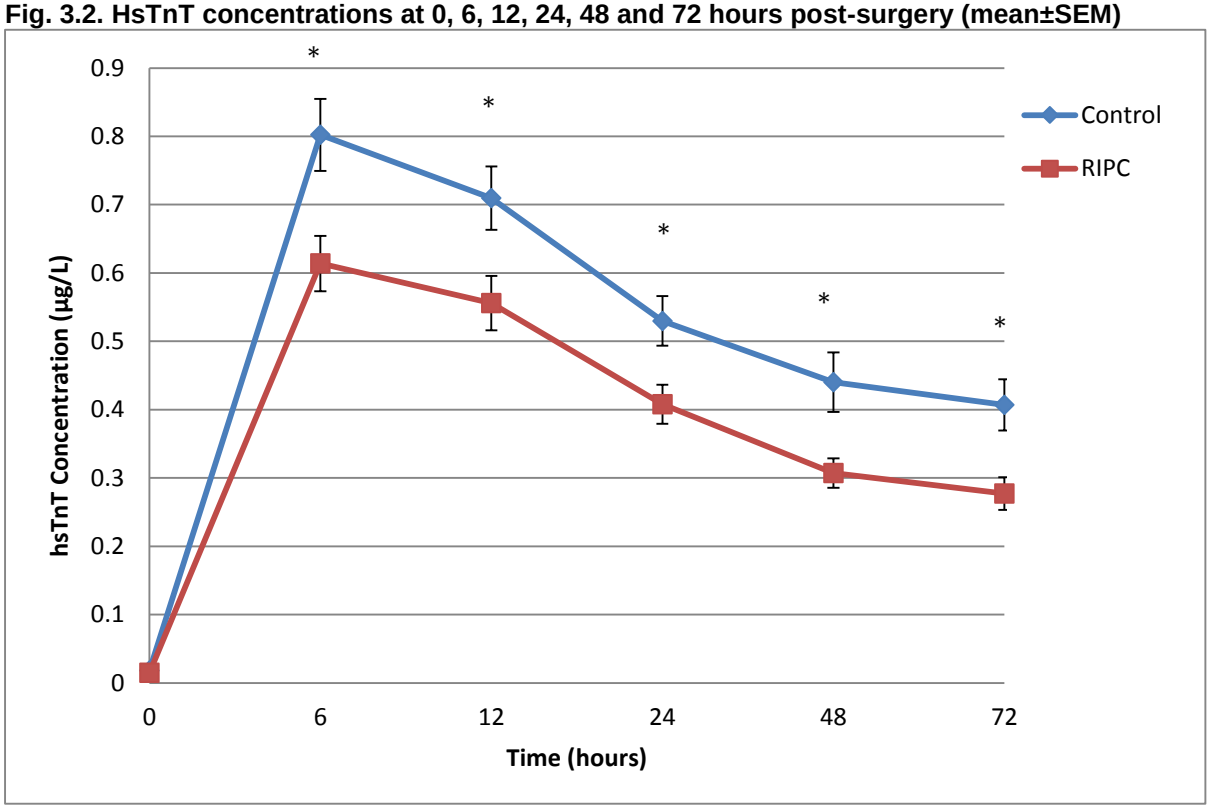
Baseline pre-operative hsTnT levels were<0.02 µg/L and not significantly different between RIPC and control patients (**Figure 3.2, Table 3.4**). Post-operative hsTnT concentration rose in both groups with a peak at 6 hours: in patients randomised to RIPC, mean hsTnT was significantly reduced at 6, 12, 24, 48 and 72 hours following surgery **(Figure 3.2, Table 3.4**). The total hsTnT release over the 72 post-operative hours, calculated as total AUC of hsTnT, was significantly lower in the preconditioned group compared to control patients and resulted in a statistically significant reduction of 25.6%, from 36.307±24.542 µg/L to 27.004±16.523 µg/L [-9.303; CI: -15.626, -2.979; p=0.004] **(Figures 3.3, Table 3.4)**.

Table 3.4. High-sensitivity Troponin-T release at the specified time point post-surgery

Endpoint	Control (n=89) (mean [SD])	RIPC (n=89) (mean [SD])	Difference (95% CI)	P value
hsTnT *(µg/L)*				
Pre-operatively	0.018 (0.019)	0.015 (0.020)	-0.003 (-0.099, 0.093)	0.310
6 hours post-operatively	0.802 (0.498)	0.614 (0.381)	-0.188 (-0.285, -0.092)	0.005
12 hours post-operatively	0.709 (0.438)	0.556 (0.376)	-0.153 (-0.250, -0.057)	0.013
24 hours post-operatively	0.529 (0.341)	0.408 (0.268)	-0.124 (-0.221 -0.027)	0.009
48 hours post-operatively	0.440 (0.408)	0.307 (0.202)	-0.137 (-0.234, -0.041)	0.007
72 hours post-operatively	0.407 (0.349)	0.277 (0.219)	-0.136 (-0.233, -0.038)	0.004
Total 72 hours AUC	36.307(24.542)	27.004 (16.523)	-9.303 (-15.626, -2.979)	0.004

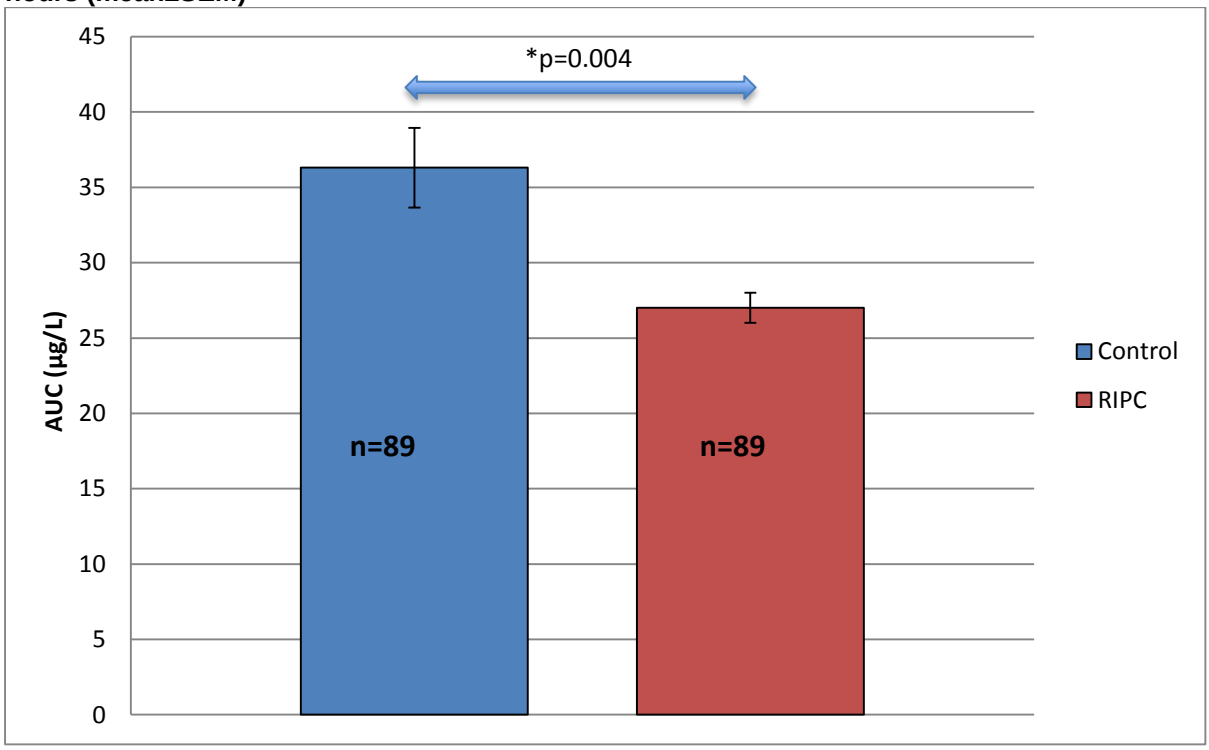
RIPC=Remote ischaemic preconditioning; SD=standard deviation; CI=confidence interval; hsTnT=high sensitivity Troponin-T

Fig. 3.2. HsTnT concentrations at 0, 6, 12, 24, 48 and 72 hours post-surgery (mean±SEM)



hsTnT=high sensitivity Troponin T; RIPC=Remote ischaemic preconditioning
• p<0.05 (unpaired Student T-Test); SEM=standard error of the mean

Fig. 3.3. Total Area under the Curve of high-sensitivity Troponin T over the 72 post-operative hours (mean±SEM)



AUC=area under the curve; RIPC=Remote ischaemic preconditioning; SEM=standard error of the mean. * Unpaired Student T-Test

3.12.1.2. RIPC protected kidney function

There was a significant improvement in urine output at 72 hours post-operatively and in total post-operative urine output in RIPC-treated patients (p=0.007 and 0.011 respectively). Creatinine levels rose in both groups with a peak at 48 hours: however, we found no significant difference in serum creatinine levels pre-operatively and at 24, 48 and 72 hours post-surgery. Importantly, both the incidence and the severity of AKI were decreased in RIPC patients and particularly we observed 19 new cases of AKI amongst control patients and 9 cases amongst preconditioned subjects: this corresponded to a 53% reduction of the total number of AKI, which was very close to statistical significance (**Table 3.5**).

Table 3.5. Summary of renal outcomes

Endpoint	Control: n=89 (mean [SD])	RIPC: n=89 (mean [SD])	Difference (95% CI)	P value
AKI score (N)				
0	70 (79%)	80 (90%)		
1	11 (12%)	6 (7%)		
2	5 (6%)	2 (2%)		
3	3 (3%)	1 (1%)		
Total number of AKI cases	19 (21%)	9 (10%)		0.063
Creatinine (mg/ml)				
Pre-operatively	87.1 (19.6)	86.1 (27.2)	-1.0 (-8.0, 6.0)	0.780
24 hours post-operatively	92.4 (30.0)	87.6 (27.0)	-4.7 (-13.2, 3.8)	0.278
48 hours post-operatively	103.3 (49.3)	92.1 (38.5)	-11.2 (-24.3, 1.9)	0.094
72 hours post-operatively	98.8 (52.6)	91.3 (43.0)	-7.5 (-21.7, 6.7)	0.306
Urine Output (ml)				
24 hours post-operatively	1989.3 (764.5)	2171.4 (626.7)	182.1 (-33.8, 398.0)	0.098
48 hours post-operatively	2144.0 (921.1)	2349.7 (848.9)	205.7 (-84.9, 496.2)	0.160
72 hours post-operatively	2026.1 (859.1)	2486.1 (832.7)	460.0 (129.3, 790.7)	0.007
Total	5858.1 (1748.7)	6706.8 (1580.2)	848.7 (197.9, 1499.5)	0.011

RIPC=remote ischemic preconditioning; sd=standard deviation; AKI= acute kidney injury

3.12.1.3. RIPC reduced the incidence of post-operative AF

In the RIPC group the incidence of new AF was significantly reduced with 22 new cases of AF in the control group and 10 new cases in preconditioned patients (**Table 3.6**), which corresponded to a statistically significant reduction of 56% of post-operative AF (p=0.03).

3.12.1.4. RIPC reduced the length of ICU stay

RIPC reduced the length of ICU stay (2.0 vs 3.0 days; p=0.04) and the total hospital stay (8.5 vs 8 days) although the latter did not reached statistical significance (p=0.094) (**Table 3.6**).

3.12.1.5. Safety of RIPC: skeletal muscle injury and clinical outcomes

Total CK release was not statistically different between control and RIPC patients (32,543±27,087 μg/L vs 36,312±19,496 μg/L [3769.6; CI -4647.0, 12186.2; p=0.38], demonstrating that the enhanced multi-limb RIPC stimulus was not associated with a significant increase in muscle injury (**Table 3.6**). Furthermore, there was no difference in major adverse clinical events at 6 weeks in patients randomised to RIPC when compared to control (**Table 3.6**): interestingly, we observed 5 deaths at six weeks in the preconditioned group and no death in control patients, although this did not reach statistically significant significance (p=0.057).

Table 3.6. Summary of study endpoints

Endpoint	Control (n=89) (mean [SD])	RIPC (n=89) (mean [SD])	Difference* (95% CI)	P value*
CK (*μg/L*)				
Total AUC	32542.8 (19495.5)	36312.3 (27087.2)	3769.6 (-4647.0, 12186.2)	0.377
Inotrope Score (mg/kg/hr)				
Post bypass	6.8 (13.5)	6.8 (15.3)	0.0 (-4.8, 4.8)	
24 hours post-operatively	11.6 (20.9)	9.4 (16.6)	-2.2 (-7.0, 2.6)	
48 hours post-operatively	8.4 (19.1)	5.5 (14.1)	-2.9 (-7.7, 2.0)	
72 hours post-operatively	5.6 (16.8)	1.7 (8.3)	-3.9 (-8.7, 1.0)	
Total	32.7 (58.8)	22.7 (42.3)	-10.0 (-25.6, 5.6)	0.206
New onset AF (N)				
	22 (25%)	10 (11%)		0.031
Length of ICU stay (days)				
	3.0 (2.0 - 4.5)**	2 .0 (1.0 – 4.0)**		0.043***
Length of hospital stay (days)				
	8.5 (7.0 – 12.0)**	8.0 (6.0 – 10.0)**		0.094***
Clinical outcomes at six weeks (N)				
Death	5 (7%)	0 (0%)		0.057
Myocardial infarction	1 (1%)	0 (0%)		0.401
Stroke	0 (0%)	1 (1%)		0.451
Revascularisation	0 (0%)	0 (0%)		1.000

RIPC=remote ischemic preconditioning; SD=standard deviation; AUC=area-under-the-curve; AF=atrial fibrillation; CK=creatine kinase; ICU=intensive care unit

*Differences, 95% CIs of the differences and p value are calculated from repeated measures regression model.

**Results shown as median (IQR).

***P Value for Mann–Whitney–Wilcoxon test.

3.13. Discussion

3.13.1. Effects of simultaneous multi-limb RIPC on PMI

This single-centre single-blinded randomised control clinical trial has demonstrated that RIPC induced by simultaneous multi-limb IR reduces PMI as evidenced by a statistically significant 25.6% reduction of hsTnT release.

Following cardiac surgery, the release of cardiac enzymes, including CK-MB (119), TnT (77-79), TnI (80, 81), has been associated with worse short and long-term clinical outcomes (77-82) with therefore a significant impact on patients' morbidity and mortality. As previously discussed, one of the potential mechanisms underlying PMI during cardiac surgery is represented by acute IRI secondary to intermittent aortic cross-clamp, ICCF or intermittent or continuous administration of cardioplegia (83). In this regard, RIPC, describing the protection provided to an organ/tissue by a stimulus generated in a remote or distant organ/tissue subjected to transient IR prior to prolonged ischaemia, offers a non-invasive strategy capable to reduce PMI in patients undergoing cardiac surgery and therefore to potentially improve their morbidity and mortality.

The concept of RIPC was first introduced by Przyklenk and colleagues (201), who found a significant reduction of MI size in dogs subjected to four-5-minutes cycles of Cx occlusion prior to 1 hour of sustained LAD ischaemia. From this "intramyocardial" application of IPC, Birnbaum et al (217) went on to demonstrate that "remote" transient ischaemia in the hind-limb, applied with a partial occlusion of the femoral artery in conjunction with rapid pacing of the gastrocnemius muscle, could reduce MI size in rabbits. Subsequently, Kharbanda and co-workers (222) were the first to apply the concept of RIPC to healthy human volunteers by inducing transient

non-invasive limb ischemia with a simple BP cuff applied to one arm and demonstrating improved endothelial function in the contralateral arm.

A significant number of RCTs have followed this pioneering discovery with often discordant outcomes **(Tables 1.10)**: Cheung et al (281) were the first to apply RIPC in a clinical setting and randomised 37 children in the context of elective corrective paediatric surgery for congenital heart defect to either control or RIPC given with 4 cycles of lower limb IR with a simple BP cuff and demonstrated decreased PMI, inotropic requirements and airway resistance in the preconditioned group. Similarly, within the context of paediatric surgery, Zhou and colleagues (307) showed that children undergoing surgical repair of simple ventricular septal defect receiving RIPC with three-5 minutes cycles of left upper arm IR 24 hours and 1 hour prior to surgery, reduced serum concentrations of IL-6, IL-8, IL-10, TNF-alpha, LDH, CK, CK-MB, and cTnI, thereby attenuating systemic inflammatory response as well as myocardial and pulmonary injury. Again, in a third interesting clinical trial in corrective paediatric surgery, Pavione and co-workers (308) applied the preconditioning stimulus (four-5 minutes cycles of lower limb IR) 24 hours prior to the operation and failed to demonstrate enhanced cardioprotection or reduced post-operative inflammatory response: nevertheless, it is possible that in this case, as previously known, the negative findings obtained could be attributed to the inferior intensity of myocardial protection provided by the second window of preconditioning compared to classic preconditioning.

However it was in the setting of adult CABG surgery that understandably RIPC found an extensive application. Here we will concentrate on the discussion of RCTs in the setting of CABG surgery with or without valve surgery whereas studies on valve surgery alone will be described in the next section.

Our research group was the first to demonstrate that RIPC reduced PMI in adult patients undergoing elective CABG surgery (282): in a pioneering single-blinded controlled RCT (282) involving 57 patients undergoing elective CABG surgery with either cardioplegia or ICCF and randomised to RIPC (three-5 minute cycles of inflation and deflation of BP cuff placed on the upper arm) or control (an un-inflated BP cuff placed on the upper arm for 30 minutes), we found that preconditioned subjects had a 43% reduction of cardiac cTnT release over the 72-hour peri-operative period compared to controls. These findings were confirmed with a further study involving 45 non-diabetic patients undergoing elective CABG with or without valve surgery and receiving cold-blood cardioplegia alone (291), thereby reflecting the more common use of this method of myocardial protection in the UK and worldwide: RIPC given with three-5 minute cycles of upper arm IR significantly reduced the 72-hour AUC of cTnT by 42.4%, demonstrating that PMI can be reduced by RIPC irrespective of the technique of myocardial preservation. The same preconditioning stimulus was applied by Ali and colleagues (445) in a study including 100 patients undergoing elective CABG for two or three-vessel CAD and similarly led to a significant reduction of post-operative CK-MB levels.

The concept of RIPC in the context of elective CABG was then extended to patients receiving antegrade cold crystalloid cardioplegia in two seminal studies by Thielmann and colleagues (293, 300). In the former (293), non-diabetic patients with triple-vessel CAD subjected to a preconditioning stimulus with three 5-minute transient upper arm IR sustained a significantly lessened PMI compared to control, with a 44.5% reduction of total 72 hr AUC of cTnI, demonstrating that RIPC can induce myocardial protection also in the context of crystalloid cardioplegia. In the latter (300), which recruited 329 patients undergoing first-time CABG surgery and is therefore the

so far largest proof-of-concept RCT on RIPC in cardiac surgery, the same preconditioning stimulus improved myocardial protection (ratio of RIPC/control for cTnI AUC was 0.83) and more importantly significantly reduced the combined endpoint of all-cause mortality, major adverse cardiac and cerebrovascular events, and repeat revascularisation: this will be further discussed in details in chapter 7.

However, recently a number of studies have failed to demonstrate significant cardioprotection provided by RIPC: within again the context of crystalloid cardioplegia the same group (295), for example, only showed a small beneficial effect of RIPC, applied 18 hours prior to elective CABG surgery with or without AVR, with TnT release only significantly reduced at 8 hours post-operatively but not at 16 or 24 hours, thereby reflecting that the late window of preconditioning is a less potent strategy than classic preconditioning. Moreover, in a third additional treatment group including subjects receiving tramadol 200 mg retard at 19:00 hours the day before surgery and at 06:00 hours on the day of the operation, PMI was significantly higher than the control group at all the time points. Similarly, Lomivorotov and co-workers (297) did not find any statistically significant benefit on PMI in patients undergoing CABG surgery with cold crystalloid cardioplegia, although importantly in this study they only measured cTnI and CK-MB pre-operatively and at 6, 24 and 48 hours post-surgery only, thereby not reflecting the true absolute post-operative release of cardiac biomarkers provided by total AUC.

Similarly, in a large trial involving 162 patients undergoing CABG surgery (286), Rahman and colleagues found no statistically significant difference between patients receiving sham or RIPC protocols (three-5 minute cycles of upper limb IR) in cTnT release, ECG changes, cardiac index, inotrope and vasoconstrictor requirement, renal impairment and lung injury. However, importantly the study included patients

undergoing elective or urgent (post-ACS) CABG surgery and it is therefore possible that the beneficial effects of RIPC might have been attenuated by the previous acute event, which could have already "preconditioned" the patients. Moreover, in this double-blinded study, patients were prepared and draped so that to obscure the visibility of both the BP cuff placed around the upper arm and the one placed around a "dummy arm" and although the correct inflation was verified though the disappearance of a pulsatile signal on a pulse oximeter, it is still possible that during the inflation and deflation phases, the cuff might have moved and that the RIPC stimulus might have not been delivered correctly. Third and importantly, a significant proportion of these patients received GTN intra-operatively which might have interfered with cardioprotection provided by RIPC: this will be also discussed in a separate section. Subsequently, Young and colleagues (296), failed to demonstrate that a standard preconditioning stimulus could improve PMI, AKI incidence or inotrope requirement in a study enrolling 96 patients undergoing high-risk cardiac surgery, including combined CABG and valve surgery, CABG surgery with LVEF<50%, "redo-operation", MV surgery, double or triple valve surgery.

Using the hypothesis that RIPC is cardioprotective under a strict anaesthetic regime, Karuppasamy and co-workers (294) recruited 54 patients undergoing elective CABG surgery and receiving the volatile anesthetic isoflurane before CPB and the intravenous anaesthetic propofol thereafter until the completion of surgery: patients subjected to a standard RIPC stimulus had no significant benefit in terms of total release of cTnI, BNP, CK-MB, cytokines and growth factors. Two other major clinical studies used a strict anaesthetic regime with similarly no significant impact on PMI (298, 299): in a first RCT involving 72 non-diabetic patients referred for elective CABG surgery (298) with crystalloid cardioplegia, anaesthesia induction was achieved in all

subjects with a combination of sufentanil, etomidate and rocuronium, and anaesthesia maintenance was ensured with either inhaled isoflurane or propofol infusion with additional sufentanil administered in both cases at the discretion of the anaesthetist: intriguingly the authors found that only with isoflurane anaesthesia RIPC could significantly reduce PMI compared to isoflurane alone, whereas no significant difference was found in patients receiving RIPC with propofol compared to those receiving propofol alone. In a further study on 55 patients undergoing CABG surgery with cold blood cardioplegia (299), anaesthesia was induced with propofol, an opioid (either fentanyl, sufentanil or romifentanil) and rocuronium and maintained with isoflurane: RIPC consisted of four-5 minutes cycles of 300mmHg cuff inflation/deflation of a BP cuff around the upper leg prior to aortic cross-clamping and did not reduce the total release of cTnT, pro-BNP, CRP, S100 protein (a marker of cerebral injury); importantly, the incidence of the composite endpoint of post-operative new arrhythmias and MI was significantly higher in the preconditioned group.

Furthermore, in a proof-of-concept study involving 130 patients undergoing off-pump CABG surgery (284), RIPC was induced by four cycles of five-minute upper limb IR and reduced total cTnI AUC by 26%, which did not reach statistical significance. However, the same group found that the combination of RIPC with RIPostC (285) in an analogous surgical setting (the same stimulus was applied twice, immediately after anaesthesia induction -RIPC- and just after completion of anastomoses -RIPostC-), could lead to a significant cTnI AUC reduction in the preconditioned group.

Additionally, a number of systematic reviews investigating the effects of RIPC on PMI with or without clinical outcomes in patients undergoing cardiac or vascular surgery or elective PCI have been conducted (289, 446-455), concluding that RIPC

reduces post-procedure myocardial damage in these subjects but does not impact on their clinical outcomes (456).

In summary, it is evident that the overall results of the numerous RCTs investigating the effects of RIPC on PMI in patients undergoing cardiac surgery have not been overwhelmingly positive. Multiple potential factors can be identified in order to provide a satisfactory explanation in this regard and these can be classified in patients' characteristics, clinical settings, anaesthetic regimes and other agents administered in the peri-operative period.

Patients' characteristics particularly involve baseline factors such as age and the presence of co-morbidities: more recently the risk profile of patients undergoing cardiac surgery has significantly changed and this is also due to the ageing population and therefore to patients with more advanced age being operated on (34). Ageing is characterised by up-regulation of Angiotensin-II receptors, activation of NADPH oxidase, increased oxidase-A activity and mitochondrial oxidative defense which ultimately increase oxidative stress and render ageing myocardium more susceptible to IRI (457): intriguingly the response of ageing myocardium to cardioprotection provided by IPC, RIPC and RIPostC as well as by pharmacological agents including opioids remains controversial (457). Moreover it has been established that the presence of comorbidities such as *diabetes*, *hypertension*, and *dyslipidaemia* may interfere with cardioprotection. In the next chapter we will discuss about the crucial role of diabetes in cardiovascular disease and its implications in the outcomes of studies evaluating the effects of RIPC on PMI in diabetic patients undergoing cardiac surgery. Intriguingly, cardioprotection provided by IPC my be lost in aging hypertensive hearts (458) and discordant results have been obtained from experimental and human

studies evaluating the effects of dyslipidaemia on myocardial IRI and more importantly on its impact on IPC, RIPC and RIPostC: there is also evidence that statins might interfere with cardioprotection though mechanisms that might be independent on their lipid-lowering actions, such plaque stabilisation, endothelial function preservation, free radical scavenging, anti-proliferative, anti-ischaemic, anti-inflammatory and anti-apoptotic effects (457). In this regard, we hypothesised that an enhanced preconditioning stimulus would be able to overcome the impaired or attenuated response to cardioprotection in patients with any of the above individual or combined conditions. Moreover and importantly, in our study we found no statistically significant difference between preconditioned and control patients in terms of age, comorbidities and concomitant medications.

In addition, **pharmacological agents** administered concomitantly with cardiac surgery have also been demonstrated to have a significant impact on cardioprotection achieved with RIPC, with particular regards to *anaesthetic drugs* and *iv nitrates* given prior to, during and post-surgery. We will discuss the role of iv GTN elsewhere. Here we will concentrate on the role of anaesthetics in preconditioning. Inhaled anaesthetics have been showed to provide myocardial protection in patients undergoing cardiac surgery (299, 459-461), either used alone or in combination with propofol (284, 298). However the use of propofol alone does not lead to cardioprotection (298), and this is probably due to the lack of action on K_{ATP} channels and its interference with ROS, which have both been implicated in mechanisms underlying RIPC (298). It is therefore possible that inhaled anaesthetics, either alone or in combination with propofol, are capable to reach the necessary threshold to induce cardioprotection and that the addition of RIPC may not provide any further benefit. In our study, propofol was given to 88% of controls and 91% of preconditioned patients during anaesthesia induction

and to 100% of patients in both groups during anaesthesia maintenance with no significant difference between the two groups; similarly, volatile anaesthetics were given to all control and RIPC patients during maintenance, in the form of either isoflurane (93% and 95% respectively) or sevoflurane (7% and 5% respectively), with again no difference between the two groups.

The **clinical setting** is another crucial aspect potentially able to interfere with cardioprotection and therefore with outcomes of some of the above-mentioned RCTs. Crucially, when patients undergoing *urgent CABG* were also included in the analysis (286), the recent occurrence of ACS might have inadvertently preconditioned these subjects and therefore attenuated cardioprotection provided by a subsequent RIPC stimulus: in this regard, our study only included patients undergoing elective cardiac surgery and with negative hsTnT at baseline. In addition, the vast majority of the clinical trials on RIPC in cardiac surgery are small proof-of-concept studies and therefore it is possible that small differences in PMI magnitude between preconditioned and control patients might have not reached statistical significance: hence we intended to maximise patient recruitment in order to increase our *sample size*. At the time of our recruitment completion, our study was the largest proof-of-concept clinical trial (n=180) investigating the effects of RIPC on PMI in an unselected population undergoing cardiac surgery. More importantly, patients undergoing elective CABG surgery sustain an overall small magnitude of PMI compared to that observed in patients presenting with STEMI (280): the detection of myocardial damage in these subjects can occur by measuring the increase of cardiac biomarkers and/or performing imaging tests as described in chapter 1. In addition, given recent developments of on-going treatment approaches of patients with CAD and more importantly the advance of

operative methods of myocardial preservation, surgical techniques and anaesthetic agents, it is possible that the additional benefit provided by RIPC to these patients might not be significant or identifiable with the current strategies. Again, we sought to overcome this potential disadvantage by delivering a higher intensity protective stimulus in order to render evident the effects of RIPC in these patients.

Therefore, from these observations, it is possible to identify in the **intensity of the preconditioning stimulus** one of the most important factors potentially able to impact on myocardial preservation in cardiac surgery: indeed an “insufficiently potent” stimulus might be associated only with a non-significant benefit compared to the one already provided by the optimisation of surgical and anaesthetic techniques as well as by pharmacological treatment or might not be able to overcome the interference due to a recent cardiac event or concomitant comorbidities and/or medications. Therefore, with our simultaneous multi-limb preconditioning stimulus we intended to “cross” the potential threshold in order to enhance cardioprotection. Moreover, in some studies the preconditioning stimulus has been delivered in different ways, by using a different number and duration of IR cycles, upper limbs versus lower limbs, dummy arms besides patients’ arms and covered by surgical gown in order to ensure blindness but at the same time leading to the possibility of misplacement of the cuff between one cycle and the other. We therefore hypothesised that PMI could be reduced by increasing the intensity of the preconditioning stimulus by applying transient simultaneous multi-limb IR: 2 cycles of 5 minutes inflation-deflation of two BP cuffs around the upper arm and the upper thigh respectively are potentially equivalent to 4 cycles of arm conditioning only, which have been successfully used in a recent trial investigating the effects of remote preconditioning in patients presenting with STEMI and undergoing PPCI (356). Only one study (306), unpublished at the time of our

patients' recruitment, had used a comparable preconditioning stimulus in patients undergoing MVR with or without concomitant TV valvuloplasty. RIPC consisted of three-5 minutes cycles of upper arm IR or three-5 minutes cycles of upper arm IR plus two 10 minutes cycles of upper leg IR. Intriguingly, whilst no difference of total cTnI was found between control and patients receiving the standard preconditioning stimulus, simultaneous multi-limb preconditioning significantly reduced mean cTnI, although no total TnI AUC was evaluated and no significant difference was observed in other outcomes.

The second considerable advantage of our simultaneous limbs preconditioning resides in the shortened time required for the delivery of the stimulus (15 minutes vs the 25 minutes used in the vast majority of clinical trials and the 35 minutes needed in Botker's work (462)) and therefore in its practicality, which represent important aspects of the patients' preparation and optimisation in the period immediately preceding cardiac surgery (by increasing the intensity of the stimulus using one limb only we would inevitably prolong the time necessary for the stimulus to be applied).

In conclusion our study demonstrated that by increasing the intensity of the preconditioning stimulus by simultaneous multi-limb IR, it is possible to reduce the magnitude of PMI in an unselected population undergoing cardiac surgery. We will show in the next section the impact of these enhanced stimulus on other study endpoints: the large multi-centre randomised control double-blinded clinical trials that are currently being undertaken will be able to potentially confirm these findings and more importantly to establish whether RIPC provides beneficial effects on clinical outcomes in patients undergoing cardiac surgery (ERICCA trial, ClinicalTrial.gov identifier: NCT01247545, and RIPHeart, ClinicalTrials.gov identifier: NCT01067703).

3.13.2. Effects of simultaneous multi-limb RIPC on AKI

AKI is a potential major complication following cardiac surgery and can significantly impact on patients' morbidity and mortality: studies have showed that it can affect up to 30% of patients and require dialysis in 1-2% of cases (364), potentially leading to an 8-fold increased death rate (365). In major vascular surgery involving the abdominal aorta, AKI has been observed in almost 10% of patients (316) and in a prospective cohort study of 4118 patients undergoing cardiac and thoracic aortic surgery (463), even changes in serum creatinine concentration higher than or equal to 0.5 ml/dL were associated with a 35% mortality rate at 30 days post-surgery. Different mechanisms have been hypothesised in order to explain renal injury following cardiac surgery and include haemodynamic effects related to CBP, metabolic factors, neuro-hormonal stimulation, systemic inflammatory response to CBP, exogenous and endogenous toxins, production of micro-emboli and oxidative stress (364). Importantly, different strategies have so far been investigated in order to preserve renal function following cardiac surgery, however with often unsatisfactory results (364), and therefore novel protective strategies are required to reduce AKI incidence and improve clinical outcomes in these patients: in this regard, RIPC has increasingly become an encouraging promise in a significant number of preclinical and clinical studies **(Table 1.16)**. In a retrospective analysis of two randomised trials primarily investigating the effects of RIPC on PMI in cardiac surgery, Venugopal et al (434) found a significant decrease of the occurrence of AKI in 38 non-diabetic patients subjected to standard RIPC stimulus: importantly this secondary analysis was small and excluded patients with diabetes and/or CKD, who are at significantly higher risk of developing AKI following cardiac surgery.

Subsequently, a prospective double-blinded RCT on 76 patients undergoing complex valve surgery (303) failed to prove statistically significant benefit of RIPC on AKI, although it demonstrated a significant reduction in CK-MB release during the post-operative 24 hours. Similarly, still within the context of valve surgery, no significant difference was found in AKI or ALI incidence between preconditioned and control patients in another study applying combined RIPC and RIPostC (305). These discrepant results between myocardial, lung and renal protection could potentially reflect different mechanisms underlying RIPC in these organs, being the heart directly and the kidneys and lungs indirectly subjected to IRI during cardiac surgery. In a further study investigating renal outcomes in 118 patients undergoing CABG (435), Zimmermann and colleagues found a reduction in the occurrence of AKI in the preconditioned group, with an absolute AKI risk reduction of 0.27 and a relative reduction of 0.43, although no difference was identified in Neutrophil Gelatinase Associated Lipocalin (NGAL) levels measured before and 3 hours after CBP. In a post-hoc analysis, the same authors also demonstrated a reduction of AKI 1-2 and of the incidence of AKI for at least 48 hours in the preconditioned patients. Interestingly, in another study by Pedersen (436) involving 105 children undergoing complex congenital heart disease, RIPC, consisting of four-5 minutes cycles of inflation to 40 mmHg above SBP of a cuff placed around the thigh, followed by 5 minutes of deflation, no difference was found in primary endpoints including AKI, initiation of dialysis, serum creatinine, eGFR, plasma cystatin C, NGAL and urinary output.

Similarly, no difference in renal outcomes was found in subsequent studies in patients undergoing CABG when off-pump surgery was performed (284), or high risk patients were included (296), or crystalloid cardioplegia (297) or strict anaesthetic regimes (298) were delivered. Thielmann et al. (293) demonstrated significantly

reduced post-operative cTnI and serum creatinine concentrations in non-diabetic subjects undergoing CABG surgery with crystalloid cardioplegia, although renal protection could not be confirmed in a more recent study by the same group (300) using a similar preconditioning stimulus in a comparable surgical setting. In the study by Rahman and colleagues (286), RIPC did not achieve statistically significant myocardial or renal protection in subjects undergoing elective or urgent CABG surgery with blood cardioplegia.

A recently published large systematic review of RCTs investigating the effects of RIPC on myocardial and renal protection (450) included 17 studies on a total of 689 preconditioned patients and 682 control subjects undergoing CABG with or without valve surgery (279, 282, 284, 286, 291, 293, 445), valve surgery alone (302), open AAA repair (292, 319, 321) and elective (339, 340) or primary PCI (355, 356). The meta-analysis concluded that RIPC patients had lower reduced PMI (standardised mean difference (SMD), 0.54; 95% CI -1.01 -0.08; p=0.01) and a lower incidence of perioperative MI (7.9% RIPC vs 13.9% placebo; RR, 0.56; 95% CI, 0.37-0.84; p=0.005); in patients undergoing AAA repair, RIPC also decreased renal injury when compared to control (SMD, 0.28; 95% CI -0.49, -0.08; p=0.007).

In our study we demonstrated a reduction of post-operative AKI incidence in preconditioned patients compared to control, with 9 new cases of AKI in the former versus 19 in the latter, which corresponded to an overall 53% reduction of the total AKI cases in our cohort population **(Table 3.5)**. However, this approached but did not reach statistical significance (p=0.063), although it is possible that it might be related to the relatively small number of patients recruited into the study. Importantly also the severity of AKI was reduced in the preconditioned group although with no statistical significance (**Table 3.5**). Encouragingly, we found a significant improvement in urine

output at 72 hours post-operatively and total post-operative urine output in RIPC-treated patients although with no significant difference in serum creatinine levels pre-operatively and at 24, 48 and 72 hours post-surgery. It is therefore possible that an enhanced RIPC stimulus may lead to an improved renal function in the post-operative period and that a large and adequately powered RCT would be able to confirm these findings. In this regard, our large multi-centre randomised double-blinded controlled ERICCA trial investigated the effect of RIPC on a number of parameters including AKI in high risk patients undergoing elective CABG±valve surgery (290) and will provide essential conclusions as to whether this simple intervention may positively impact on these subjects' clinical outcomes. Noticeably, another important RCT undertaken in the UK involving patients undergoing live-donor-related renal transplantation, found that limb RIPC of both donors and recipients preserved the transplanted kidney function at 6 months in recipients of live-donor related renal transplantation and therefore showed the protective function of RIPC on transplanted renal grafts (REPAIR trial: 'Renal Protection Against Ischaemia-Reperfusion in Transplantation', ISRCTN30083294).

In conclusion, similarly to the field of myocardial protection, clinical trials on renal outcomes with RIPC following cardiac surgery, major vascular procedures and elective PCI have often led to discrepant results and therefore larger multicentre studies are required in order to determine whether the application of RIPC to these clinical settings will translate into improvement of kidney protection – as well as cardioprotection- and therefore of morbidity and mortality in these patients.

3.13.3. Effects of simultaneous multi-limb RIPC on new onset of post-operative AF

In our study, RIPC reduced the incidence of post-operative AF by 55% compared to control. New onset AF occurs in 30-50% of patients following cardiac surgery (444), with an incidence of 30% post-CABG surgery, 40% following valve surgery and 50% further to CABG plus valve surgery (444). Importantly AF is also associated with increased rates of death, thrombo-embolic events, LV failure, prolonged hospitalization, reduced quality of life and poor exercise capacity (444). The aetiology of post-operative AF is multi-factorial and can be related to common pathogenetic factors to the general populations, such as age, CAD, rheumatic heart disease, thyrotoxicosis, cardiomyopathy, MV disease, haemochromatosis, infection, but also and more importantly to parameters directly or indirectly related to the surgery in itself, including hypovoloemia, electrolyte imbalance, central venous catheters, prolonged aortic cross-clamp times, increased automaticity, increased sympathetic tone (also due to the use of inotropes) and importantly acute myocardial IRI (444).

Rahman et al (286) failed to demonstrate any beneficial effect of RIPC on AF incidence following cardiac surgery, the potential reasons for which have already been discussed. Similarly no difference was found in patients undergoing valve surgery alone (305), or CABG surgery when RIPC was applied during isoflurane inhalation (299) or in children receiving corrective repair for congenital heart disease when RIPC was delivered with four-5 cycles of lower limb IR 24 hours before the operation (in this case the authors showed no statistical difference in the total rate of post-operative arrhythmias) (308).

In our cohort, RIPC reduced the incidence of new post-operative AF by 55%, with 10 new cases in the RIPC group and 22 in the control group, which corresponded

to 25% and 11% of patients respectively, a reduction which we found to be statistically significant (p=0.031) (**Table 3.6**). It is possible that, given that IRI is one of the most relevant pathogenetic factors implicated in the mechanisms of AF following cardiac surgery, RIPC may have decreased the incidence of post-operative AF by protecting the myocardium against acute IRI. This may imply that compared to the above-mentioned studies, by increasing our preconditioning stimulus and therefore by achieving a significant PMI reduction, we have reached the potential threshold capable to enhance cardioprotection and its consequences, including amongst others the decreased incidence of new onset post-operative AF.

Additionally, a 55% reduction in post-operative AF is extremely encouraging as it would also be expected to have beneficial effects on short and long-term clinical outcomes resulting in improved patient morbidity and mortality and reduced healthcare costs. This again leads to the significant relevance of large RCTs to investigate the effects of RIPC on new onset of post-operative AF in the context of cardiac surgery in order to better understand the mechanisms underlying its occurrence and more importantly the impact of PMI reduction on its incidence and on subsequent clinical outcomes. In this regard, the on-going large multi-centre RICO trial is also investigating the effects of RIPC on AF incidence in CABG patients (464).

3.13.4. Effects of simultaneous multi-limb RIPC on ICU/hospital stay

In our cohort, we were able to find a statistically significant difference between the two treatment groups in term of length of ICU stay, with 2 versus 3 days of total ICU stay in RIPC and control groups respectively (p=0.043) (**Table 3.6**). Previous studies including children undergoing corrective cardiac surgery (306-308, 436), adults receiving valve surgery alone (301, 302, 305, 306) or elective CABG surgery with crystalloid cardioplegia (293, 300), elective or urgent CABG (286) failed to demonstrate this beneficial effect. However, similarly to these studies, we found no significant difference in total hospital stay (p=0.094).

We hypothesise that in our RCT, ICU stay reduction could be directly or indirectly related to the enhanced preconditioning stimulus delivered and particularly to:

1) the reduction in PMI, which can potentially improve cardiac function thereby limiting the period of time for the patient to require intensive care management;
2) the decrease in new onset of post-operative AF, therefore reducing the potential haemodynamic instability that could arise subsequently to poorly controlled AF;
3) the reduction of AKI incidence and severity, although this only approached but did not reach statistical significance.

The importance of this finding in our single-centre study has the potential to be clinically relevant as clearly a reduction of ICU total stay may have a significant impact on patients short and long term clinical outcomes and subsequently on NHS resources and costs. However, it is also relevant to specify again that the reduction of ICU stay did not result in a significant decrease of total hospital stay: our ERICCA trial will once again give us clarification on such an intriguing result.

3.13.5. Effects of simultaneous multi-limb RIPC on other secondary end-points

In addition to the above findings, our study did not show any significant difference between preconditioned and control patients in terms of **inotrope requirement** (**Table 3.6**). This also confirms similar outcomes from previous studies (286, 297, 300, 302, 305, 306, 436). Importantly, RIPC has been associated with increased inotropic requirement in high-risk patients undergoing double or triple valve surgery, MV surgery, CABG plus valve surgery or CABG with pre-operative LVEF<50% (296) and only two studies (281, 301) have so far reported beneficial effects of RIPC on this outcome: the former was the first fundamental application of RIPC in the clinical setting and showed that lower limb IR also induced myocardial and lung protection in corrective paediatric surgery (281); the latter included valve surgery alone and concluded that both the total of number of patients requiring inotropes and the total inotrope dose needed were significantly lower in preconditioned subjects (301).

Crucially, we also find that our enhanced preconditioning stimulus did not result in significant **muscle damage**: one could argue that transient simultaneous multi-limb IRI by inflating two BP cuffs, one around the upper arm and one around the upper thigh, could lead to an increased limb skeletal muscle injury. For this reason, we excluded patients with known significant PAD of upper and/or lower limbs. We found no statistically significant difference in total CK AUC (32542.8±19495.5 μg/L in controls versus 36312.3±27087.2 μg/L in preconditioned patients [3769.6; CI -4647.0, 12186.2;p=0.377] (**Table 3.6**) (465), therefore proving that our increased RIPC stimulus does not results in relevant muscle damage and can safely be delivered in the absence of significant PAD.

Finally, despite the non-statistically significant incidence of 5 deaths in the preconditioned group at six weeks post-surgery versus no death in the control group, we found no relevant difference in short term **clinical outcomes** between control and RIPC subjects (**Table 3.6**): this will be further discussed in chapter 5, where we will also explain the potential impact of an increased RIPC on clinical outcomes at one year, the primary study endpoint of our ERICCA trial (290).

3.13. Post-hoc subgroup analyses

Our relatively large cohort size allowed us to perform a series of retrospective sub-group analyses to determine whether the protective effects of our enhanced preconditioning stimulus we have found in the principal study would also apply in more specific settings, based on the technique of myocardial preservation utilised, the type of operation and crucially the presence or absence of diabetes. However, it is also crucial to highlight that, whilst our principal study was adequately powered for the primary end-point of PMI, findings deriving from these retrospective sub-group analyses are only suggestive of potential outcomes and certainly require larger RCTs to be confirmed.

3.13.1 Multi-limb RIPC and techniques of myocardial preservation: effects on cardioprotection in patients undergoing cardiac bypass surgery using either cardioplegia or ICCF

We have so far described the effects of an enhanced RIPC stimulus in an unselected cohort of patients undergoing cardiac surgery and demonstrated that simultaneous transient multi-limb IR significantly reduces PMI, new post-operative AF incidence and total ICU stay length with no significant impact on inotrope requirement, hospital stay duration and clinical outcomes at six weeks and a trend towards statistical significance of AKI incidence reduction. As previously discussed, cardioplegia is the prevalent technique of myocardial preservation in cardiac surgery (30, 466, 467), although studies have proved that the magnitude of cardioprotection provided by cardioplegia or ICCF is essentially equivalent, as the more significant cellular protective effects of cardioplegia are balanced by the more prolonged cross-

clamp times (89-93) compared to ICCF. Also, interestingly so far only one small RCT has demonstrated the cardioprotective effects of IPC in patients undergoing CABG using ICCF (468) and no published study has been conducted to evaluate the potential impact of RIPC in these subjects. We therefore conducted a retrospective analysis in order to establish whether the same enhanced preconditioning stimulus may enhance cardioprotection irrespective of the technique of myocardial preservation used.

3.13.1.1. Results

From a total of 178 patients recruited into our study, 148 subjects received cardioplegia and the remaining 30 ICCF: in the first subgroup, 73 were randomised to the sham protocol and 75 to RIPC (82% and 84% respectively in the sham and RIPC groups), whereas in the ICCF subgroup we had 16 control and 14 RIPC patients (18% and 16% respectively, **Table 3.7)**. We therefore wish to emphasise again that this retrospective analyses are clearly underpowered and, for this reason, findings should be considered as suggestive of potential effects of RIPC in these setting and will require further confirmation with larger RCTs studies. We found no difference in terms of patients' baseline characteristics between control and preconditioned patients within both cardioplegia and ICCF subgroups **(Tables 3.7)**. With regards to parameters related to surgery, again no statistically significant difference was observed within the ICCF groups, whereas in patients receiving cardioplegia the only significant difference between the two intervention groups was interestingly the use of intra-operative GTN, which was higher in the control group (51 versus 41 patients; p=0.037, **Tables 3.8**): we will discuss later in more details about the significance of intravenous GTN peri-operatively.

Table 3.7. Patient baseline characteristics in patients receiving cardioplegia or ICCF

Patients	Cardioplegia			Cross-clamp fibrillation		
	Control (n=73)	RIPC (n=75)	P value	Control (n=16)	RIPC (n=14)	P value
Age (*years*)	67±10	66±10	0.334	62±10	60±10	0.434
Gender			0.848			0.103
Male	55 (75.3%)	58 (77.3%)		12 (75.0%)	14 (100.0%)	
Female	18 (24.7%)	17 (22.7%)		4 (25.0%)	0 (0.0%)	
Ethnicity			0.471			0.547
Caucasian	61 (83.6%)	58 (77.3%)		13 (81.3%)	13 (92.9%)	
Asian	8 (11.0%)	11 (14.7%)		2 (12.5%)	1 (7.1%)	
Afro-Caribbean	3 (4.1%)	6 (8.0%)		1 (6.3%)	0 (0%)	
Chinese	1 (1.4%)	0 (0%)		0 (0%)	0 (0%)	
BMI	28.0±5.5	28.7±7.5	0.563	30.4±4.9	29.4±4.3	0.573
SBP *(mmHg)*	130.3±18.4	129.5±16.6	0.799	128.3±16.9	126.2±10.0	0.697
DBP *(mmHg)*	70.2±8.7	71.1±9.7	0.579	73.6±10.6	69.5±7.6	0.279
HR *(bpm)*	69.3±11.9	66.4±9.7	0.110	65.9±10.4	65.9±10.4	0.486
Smoking History			0.870			0.542
Smoker	8 (11.0%)	9 (12.0%)		4 (25.0%)	2 (14.3%)	
Ex-smoker	44 (60.3%)	42 (56.0%)		8 (50.0%)	6 (42.9%)	
Non-smoker	21 (28.8%)	24 (32.0%)		4 (25.0%)	6 (42.9%)	
Family History of IHD	44 (60.3%)	54 (72.0%)	0.165	13 (81.3%)	10 (71.4%)	0.526
NYHA Class	2.88±0.9	2.58±0.8	0.036	2.29±0.9	2.23±0.8	0.872
CCS Class	2.17±1.2	2.25±1.1	0.661	2.43±0.85	2.85±0.89	0.227
LVEF			0.261			0.976
>50%	58 (79.5%)	57 (76.0%)		12 (75.0%)	10 (71.4%)	
30%-50%	14 (19.2%)	13 (17.3%)		3 (18.8%)	3 (21.4%)	
<30%	1 (1.4%)	5 (6.7%)		1 (6.3%)	1 (7.1%)	
Co-morbidities						
Diabetes mellitus	18 (24.7%)	23 (30.7%)	0.414	6 (37.5%)	5 (45.5%)	0.919
Hypertension	57 (78.1%)	56 (74.7%)	0.625	13 (81.3%)	9 (64.3%)	0.295
Hypercholesterolemia	49 (67.1%)	56 (74.7%)	0.367	15 (93.8%)	12 (85.7%)	0.464
Atrial Fibrillation	16 (21.9%)	9 (12.0%)	0.247	0 (0%)	1 (7.1%)	0.277
Previous MI	20 (27.4%)	23 (30.7%)	0.719	3 (18.8%)	5 (35.7%)	0.417
Previous PCI	10 (13.7%)	8 (10.7%)	0.622	1 (6.3%)	3(21.4%)	0.222
Previous CVA/TIA	9 (12.3%)	5 (6.6%)	0.138	0 (0%)	0 (0%)	1.000
Previous Cardiac Surgery	2 (2.8%)	4 (5.3%)	0.615	0 (0%)	0 (0%)	1.000
Other comorbidities	6 (8.2%)	3 (4.1%)	0.550	1 (6.7%)	0 (0%)	0.452
Peripheral Arterial Disease	5 (6.8%)	1 (1.3%)	0.114	1 (6.3%)	0 (0%)	0.341
Drug History	54 (74.0%)	59 (80.8%)	0.609	12 (85.8%)	13 (81.3%)	0.289
Aspirin	24 (32.8%)	18 (24.7%)	0.207	3 (21.4%)	4 (30.8%)	0.580
Clopidogrel/Prasugrel	9 (12.3%)	5 (6.9%)	0.529	0 (0%)	1 (7.1%)	0.290
Warfarin	43 (58.9%)	45 (61.6%)	0.588	11 (78.6%)	12 (92.3%)	0.315
Beta-blocker	26 (35.6%)	20 (27.4%)	0.099	3 (21.4%)	2 (15.4%)	0.686
Calcium Channel Blocker	14 (80.8%)	13 (82.2%)	0.332	13 (92.8%)	12 (82.3%)	0.985
Statin	51 (69.9%)	58 (65.8%)	0.383	8 (50.0%)	8 (69.2%)	0.714
ACE-I/ARB	10 (13.7%)	10 (13.7%)	0.362	2 (14.3%)	2 (15.4%)	0.936
Long acting nitrates						
Antidiabetics						
Insulin	5 (6.9%)	5 (6.8%)	0.574	2 (14.3%)	3 (23.1%)	0.557
Biguanide	13 (17.8%)	17 (23.0%)	0.480	1 (7.1%)	0 (0.0%)	0.617
Sulphonylurea	9 (12.3%)	8 (8.6%)	0.802	2 (14.3%)	0 (0.0%)	0.157
Diuretics	23 (31.5%)	26 (35.6%)	0.861	4 (28.6%)	5 (38.5%)	0.156

ICCF=intermittent cross-clamp fibrillation; RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA= Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB= Angiotensin receptor blocker; LVEF= left ventricular ejection fraction.

Table 3.8. Details of surgical procedure in patients receiving cardioplegia or ICCF

Patients	Cardioplegia			Cross-clamp fibrillation		
	Control (n=73)	RIPC (n=75)	P value	Control (n=16)	RIPC (n=14)	P value
Indication for Surgery			0.539			0.743
Angina	31 (42.5%)	28 (37.3%)		13 (81.3%)	12 (85.7%)	
Myocardial Infarction	9 (12.3%)	17 (22.7%)		3 (18.8%)	2 (14.3%)	
Valve Disease	23 (31.5%)	23 (30.7%)		0 (0.0%)	0 (0.0%)	
Angina and Valve Disease	7 (9.6%)	4 (5.3%)		0 (0.0%)	0 (0.0%)	
MI and Valve Disease	1 (1.4%)	2 (2.7%)		0 (0.0%)	0 (0.0%)	
Infective Endocarditis	2 (2.7%)	1 (1.3%)		0 (0.0%)	0 (0.0%)	
EuroSCORE	3.95±2.03	4.03±2.54	0.830	2.69±1.70	1.93±2.2	0.296
Additive perioperative risk			0.464			0.388
Low (EuroSCORE 0-2)	18 (24.7%)	20 (26.7%)		8 (50.0%)	9 (64.3%)	
Medium (EuroSCORE 3-5)	40 (54.8%)	34 (45.3%)		7 (43.8%)	4 (28.6%)	
High (EuroSCORE >5)	15 (20.5%)	21 (28.0%)		1 (6.3%)	1 (7.1%)	
Bypass-time (min)	104.4±33.12	91.99±32.73	0.122	77.2±21.9	77.3±15.1	0.992
Cross-clamp time (min)	70.56±24.44	66.49±26.41	0.336	33.0±7.5	35.3±7.1	0.424
Operation			0.980			1.00
CABG alone	38 (52.1%)	43 (57.3%)		16 (100%)	14 (100%)	
AVR alone	15 (20.5%)	14 (18.7%)		0 (0.0%)	0 (0.0%)	
CABG+AVR	10 (13.7%)	9 (12.0%)		0 (0.0%)	0 (0.0%)	
MVR or MV Repair	9 (12.3%)	8 (10.7%)		0 (0.0%)	0 (0.0%)	
AVR+MVR	1 (1.4%)	1 (1.3%)		0 (0.0%)	0 (0.0%)	
Number of grafts			0.802			0.587
One	4 (5.5%)	5 (6.7%)		0 (0.0%)	0 (0.0%)	
Two	16 (21.9%)	14 (18.7%)		3 (18.8%)	1 (7.1%)	
Three	19 (26.0%)	26 (34.7%)		10 (62.5%)	9 (64.3%)	
Four	9 (12.3%)	7 (9.3%)		3 (18.8%)	4 (28.6%)	
Anesthetic agents						
Induction						
Anti-nicotinic agents			0.149			0.385
Rocuronium	56 (78.9%)	65 (90.3%)		12 (92.3%)	11 (84.6%)	
Pancuronium	13 (18.3%)	5 (6.9%)		1 (7.7%)	1 (7.7%)	
Vecuronium	2 (2.8%)	2 (2.8%)		0 (0.0%)	1 (7.7%)	
Midazolam	38 (53.5%)	28 (38.9%)	0.094	7 (53.8%)	5 (38.5%)	0.431
Etomidate	6 (8.3%)	7 (9.7%)	0.771	2 (14.3%)	0 (0.0%)	0.157
Fentanyl	73 (100%)	75 (100%)	1.000	16 (100%)	14 (100%)	1.00
Propofol	64 (88.9%)	64 (88.9%)	1.000	12 (85.7%)	14 (100%)	0.157
Maintenance						
Propofol	73 (100%)	75 (100%)	1.000	16 (100%)	14 (100%)	1.000
Volatile Anesthetics						
Isoflurane	68 (93.1%)	73 (97.2%)	1.000	15 (92.9%)	12 (84.6%)	0.265
Sevoflurane	6 (7.1%)	4 (4.8%)	0.441	1 (7.1%)	2 (4.8%)	0.805
Intra-operative GTN	51 (72.9%)	41 (55.4%)	0.037	14 (87.5%)	12 (85.7%)	0.886

ICCF=intermittent cross-clamp fibrillation; RIPC=Remote Ischemic Preconditioning; MI=myocardial infarction; CABG=Coronary artery bypass graft; AVR=Aortic valve replacement; MVR=Mitral valve replacement; MV=Mitral valve; GTN=glyceryl trinitrate.

Importantly, whilst the cardioplegia group comprised patients undergoing CABG and/or valve surgery, in the ICCF group we only had patients receiving CABG: this was also associated with significantly higher EuroSCORE, bypass times and cross-clamp times in the cardioplegia group compared to cross-clamp group (p<0.001, **Table 3.9**).

Table 3.9. Comparison of EuroSCORE, cardio-pulmonary and cross-clamp times between cardioplegia and ICCF groups

Parameters	Cardioplegia (n=148)	ICCF (n=30)	Difference (95% CI)	P value
EuroSCORE	3.99 (2.30)	2.33 (1.95)	1.65 (0.77, 2.54)	<0.001
Cardio-pulmonary bypass time	96.18 (33.09)	77.25 (18.52)	18.93 (6.21, 31.66)	<0.001
Cross-clamp time	68.49 (25.45)	34.19 (7.25)	34.31 (24.55, 44.07)	<0.001

ICCF=intermittent cross-clamp fibrillation; CI=confidence interval

For this reason it is clear that the cardioplegia group had the potential of being subjected to a more significant PMI than the patients receiving ICCF: therefore in this sub-group analysis we were intrigued to know whether our multi-limb preconditioning stimulus may protect patients receiving either the techniques of myocardial preservation and particularly those higher risk patients receiving cardioplegia.

In the cardioplegia group, we found no difference in baseline hsTnT level, however crucially hsTnT concentrations were significantly lower in preconditioned patients at 6, 12, 24, 48, and 72 hours post-surgery **(Table 3.11, Fig. 3.4)** and more importantly, the total 72 hours AUC of hsTnT was 37.089±25.730μg/L versus 27.942±17.386μg/L in control and preconditioned patients respectively, with a

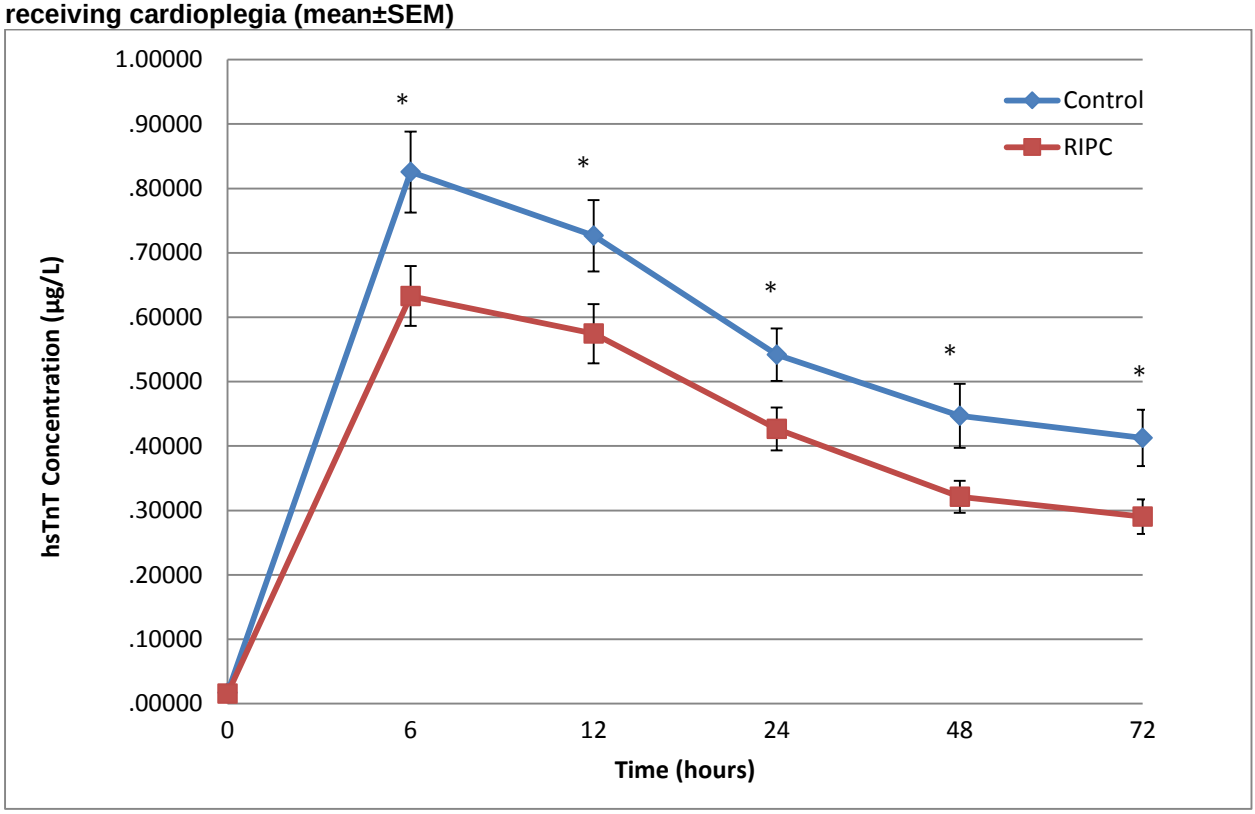
significant AUC reduction of approximately 25% [9.146; 1.861-16.433; p=0.014] **(Table 2.11, Fig. 2.5)**. Crucially, in the ICCF group mean hsTnT levels were similar pre-operatively between RIPC and control groups and significantly lower in preconditioned patients at 6, 12 and 72 hours post-surgery, with a trend towards statistical significance at 24 and 48 hours **(Table 2.11, Fig. 2.6)** and total hsTnT AUC was 32.885±18.771 μg/L in preconditioned patients and 20.692±6.039 μg/L in sham subjects, which corresponded to a statically significant reduction of 37% [12.192; CI 0.066, 24.319; p=0.044] **(Table 2.11, Fig. 2.7)**

Table 3.10. High-sensitivity Troponin-T release at the specified time point post-surgery in patients receiving cardioplegia or ICCF

Endpoint		Control Cardioplegia:n=73 ICCF: n=16 (mean (sd))	RIPC Cardioplegia:n=75 ICCF: n=14 (mean (sd))	Difference (95% CI)	P value
hsTnT *(μg/L)*					
Pre-operatively	Cardioplegia	0.017 (0.018)	0.016 (0.021)	0.001 (-0.005,0.008)	0.656
	ICCF	0.022 (0.022)	0.011 (0.013)	0.011 (-0.003, 0.024)	0.126
6 hours post-operatively	Cardioplegia	0.826 (0.537)	0.633 (0.404)	0.192 (0.038, 0.035)	0.015
	ICCF	0.696 (0.235)	0.511 (0.199)	0.185 (0.021, 0.349)	0.029
12 hours post-operatively	Cardioplegia	0.727 (0.474)	0.574 (0.399)	0.152 (0.009, 0.294)	0.036
	ICCF	0.631 (0.195)	0.457 (0.187)	0.173 (0.029, 0.317)	0.020
24 hours post-operatively	Cardioplegia	0.542 (0.349)	0.427 (0.286)	0.115 (0.01, 0.219)	0.030
	ICCF	0.476 (0.305)	0.309 (0.102)	0.166 (-0.003, 0.336)	0.054
48 hours post-operatively	Cardioplegia	0.447 (0.423)	0.321 (0.214)	0.126 (0.017, 0.235)	0.026
	ICCF	0.409 (0.339)	0.228 (0.080)	0.182 (-0.016, 0.379)	0.071
72 hours post-operatively	Cardioplegia	0.413 (0.366)	0.290 (0232)	0.123 (0.022, 0.222)	0.019
	ICCF	0.381 (0.271)	0.189 (0.066)	0.192 (0.043, 0.341)	0.014
Total 72 hours AUC	Cardioplegia	37.089 (25.730)	27.942 (17.386)	9.146 (1.861, 16.433)	0.014
	ICCF	32.885 (18.771)	20.692 (6.039)	12.192 (0.066, 24.319)	0.049

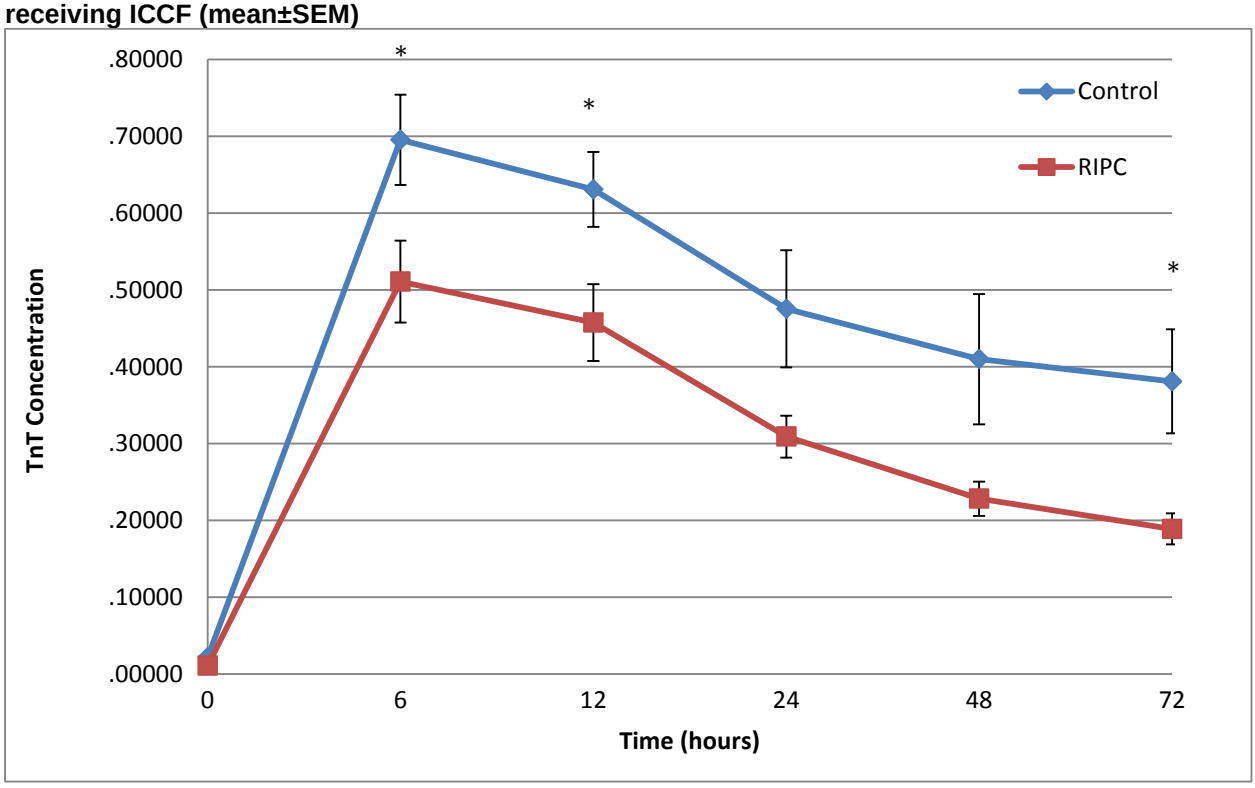
RIPC=Remote ischaemic preconditioning; SD=standard deviation; CI=confidence interval; hsTnT=high sensitivity Troponin-T; ICCF=intermittent cross-clamp fibrillation; AUC=area-under-the-curve

Fig. 3.4. High-sensitivity Troponin T at 0, 6, 12, 24, 48 and 72 hours post-surgery in patients receiving cardioplegia (mean±SEM)



hsTnT=high sensitivity troponin T; RIPC=Remote ischaemic preconditioning; SEM=standard error of the mean; *p<0.05 (unpaired Student T-Test)

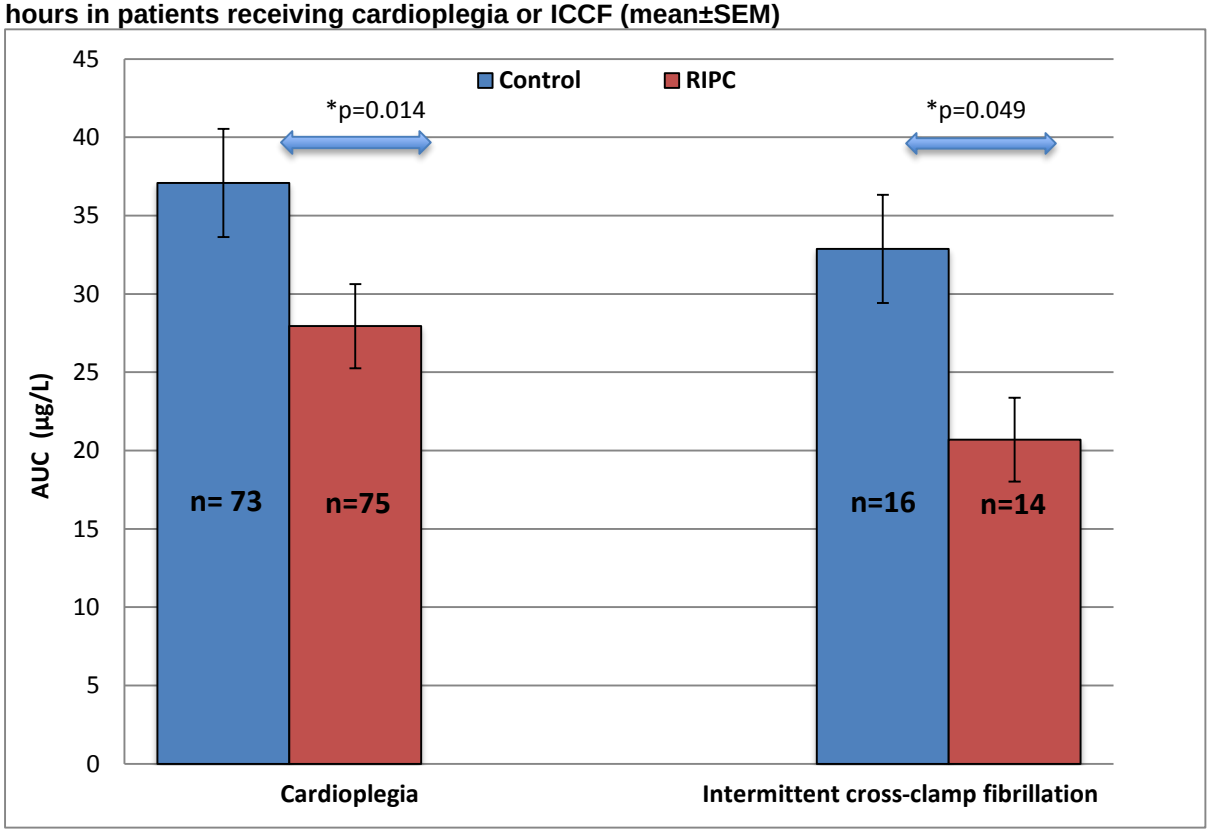
Fig. 3.5. High-sensitivity Troponin T at 0, 6, 12, 24, 48 and 72 hours post-surgery in patients receiving ICCF (mean±SEM)



ICCF= intermittent cross-clamp fibrillation; hsTnT=high sensitivity troponin T; RIPC=Remote ischaemic preconditioning; SEM=standard error of the mean. *p<0.05 (unpaired Student T-Test)

Interestingly, in contrast with the findings arising from the main analysis in the previous section, we found no statistically significant difference of any of the secondary endpoints in ether the cardioplegia or ICCF groups **(Tables 3.12 and 3.13)**. In particular in the cardioplegia group, we observed a higher incidence of post-operative AKI in the RIPC group, however this did not reach statistical significance. Conversely, we found an important reduction of new-onset AF occurrence and total inotrope requirement, although once again statistical analysis showed no significance difference. Similarly, in the ICCF group, despite an important reduction of inotrope score, this did not reach statistical significance: interestingly, no death, stroke, repeat revascularisation or myocardial infarction was observed in ICCF patients at six weeks follow-up.

Fig. 3.6. Total Area under the Curve of high-sensitivity Troponin T over the 72 post-operative hours in patients receiving cardioplegia or ICCF (mean±SEM)



RIPC=Remote ischaemic preconditioning; AUC=area under the curve; SEM=standard error of the mean. * Unpaired Student T-Test

Table 3.11. Summary of major secondary endpoints in patients receiving cardioplegia*

Endpoint	Control (n=73) (mean (sd))	RIPC (n=75) (mean (sd))	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	30389.7 (13465.8)	35747 (24932.9)	-5357 (-12998.2, 2283.4)	0.158
Creatinine (mg/ml)				
Pre-operatively	86.8 (19.5)	84.5 (25.9)	2.3 (-5.2, 9.8)	0.542
24 hours post-operatively	89.0 (25.9)	87.3 (27.3)	1.8 (-6.9, 10.4)	0.678
48 hours post-operatively	97.3 (38.3)	90.7 (38.6)	-6.6 (-5.9, 19.0)	0.300
72 hours post-operatively	94.3 (48.1)	90.44 (41.9)	3.9 (-10.8, 18.6)	0.601
Urine Output (ml)				
24 hours post-operatively	1998.5 (762.1)	2150.6 (554.2)	-152.1 (-376.6, 72.3)	0.182
48 hours post-operatively	2162.5 (922.9)	2309.7 (816.9)	-147.2 (-456.6, 162.2)	0.350
72 hours post-operatively	1944.2 (786.0)	2476.7 (877.7)	-532.5.0 (-892.2, -172.8)	0.004
Total	5829.9 (1706.2)	6687.1 (1636.2)	-857.2.7 (-1578.2.9, -136.6)	0.020
AKI score				
0	61	68		0.099
1	7	4		
2	2	1		
3	2	0		
Acute Kidney Injury	3 (5%)	6 (8%)		0.207
Inotrope score				
Post bypass	7.4 (13.9)	7.7 (16.3)	-0.3 (-5.3, 4.7)	0.905
24 hours post-operatively	11.9 (20.7)	9.8 (17.0)	2.1 (-4.2, 8.4)	0.510
48 hours post-operatively	7.1 (14.6)	5.9 (14.8)	1.1 (-3.8, 6.0)	0.652
72 hours post-operatively	3.9 (10.6)	1.5 (8.3)	-3.9 (-8.0, 0.1)	0.128
Total	30.7 (49.2)	24.1 (43.8)	6.6 (-8.9, 22.1)	0.405
New onset AF	17 (23%)	9 (12%)		0.071
Length of ICU stay (days)	3.0 (2.0 - 4.0)**	2.0 (1.0 – 3.5)**		0.846***
Length of hospital stay (days)	9.0 (7.0 – 12.0)**	8.0 (6.0 – 10.5)**		0.256***
Clinical outcomes at six weeks				
Death	1	0		0.353
Myocardial infarction	1	0		0.309
Stroke	0	2		0.225
Revascularization	1	0		0.309

Table 3.12. Summary of study endpoints in patients receiving ICCF*

Endpoint	Control (n=16) (mean [SD])	RIPC (n=14) (mean [SD])	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	48844.29 (42601.95)	40995.43 (43496.62)	7848.86 (-42290.09, 57987.81)	0.739
Creatinine (mg/ml)				
Pre-operatively	88.2 (20.9)	94.4 (33.0)	-6.2 (-26.7, 14.2)	0.536
24 hours post-operatively	107.5 (42.1)	89.6 (26.6)	17.9 (-8.9, 44.7)	0.182
48 hours post-operatively	130.6 (79.2)	99.5 (38.7)	31.1 (-15.2, 77.4)	0.177
72 hours post-operatively	119.4 (67.7)	96.0 (49.7)	23.4 (-21.6, 68.4)	0.296
Urine Output (ml)				
24 hours post-operatively	1941.9 (806.7)	2283.5 (949.9)	-341.6 (-1054.9, 371.7)	0.333
48 hours post-operatively	2033.1 (951.3)	2582.2 (1029.1)	-549.1 (-1453.7, 355.5)	0.219
72 hours post-operatively	2456.0 (1138.4)	2526.5 (640.1)	-70.5 (-968.5, 827.5)	0.870
Total	6006.3 (2080.1)	6791.6 (1387.3)	-785.4 (-2656.2, 1085.5)	0.378
AKI score				
0	10 (71.4%)	12 (85.7%)		0.789
1	1 (7.1%)	1 (7.1%)		
2	2(4.5%)	1 (7.1%)		
3	1 (7.1%)	0 (0.0%)		
Acute Kidney Injury	2 (16.8%)	0 (0.0%)		0.171
Inotrope score				
Post bypass	4.029 (11.923)	2.031 (4.958)	1.998 (-5.347, 9.342)	0.560
24 hours post-operatively	10.107 (22.884)	7.192 (13.879)	2.915 (-12.236, 18.066)	0.695
48 hours post-operatively	14.928 (33.571)	2.923 (9.673)	12.005 (-7.921, 31.932)	0.219
72 hours post-operatively	13.543 (33.128)	2.590 (8.478)	10.953 (-8.594, 30.499)	0.251
Total	42.536 (94.659)	14.736 (32.703)	27.799 (-29.252, 84.852)	0.325
New onset AF	5 (31.3%)	1 (7.1%)		0.101
Length of ICU stay (days)	3.0 (1.0-7.5)**	2.0 (1.0 – 4.0)**		0.245***
Length of hospital stay (days)	8.0 (6.0 – 10.5)**	8.0 (6.0 – 9.0)**		0.237***
Clinical outcomes at six weeks				
Death	0 (0.0%)	0 (0.0%)		1.000
Myocardial infarction	0 (0.0%)	0 (0.0%)		1.000
Stroke	0 (0.0%)	0 (0.0%)		1.000
Revascularization	0 (0.0%)	0 (0.0%)		1.000

*List of abbreviations
ICCF=intermittent cross-clamp fibrillation; RIPC=Remote ischemic preconditioning; CK=Creatinine Kinase; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve
**Results shown as median (inter-quartile range); *** P-value for Mann-Whitney-Wilcoxon test

3.13.1.2. Discussion

In the previous section we demonstrated that an enhanced preconditioning stimulus reduces PMI, incidence of new onset AF and total ICU stay in an unselected cohort of patients undergoing CABG surgery and/or valve surgery using either cardioplegia or ICCF as the technique of myocardial preservation. Importantly in our cohort comprising a total of 178 patients, 83% of subjects received cardioplegia and 17% ICCF. Crucially, in this retrospective analysis we have found that, by applying simultaneous multi-limb IR, the total hsTnT AUC was reduced from 37.089±25.730 µg/L to 27.942±17.386 µg/L [9.146; 1.861-16.433;p=0.014] in the cardioplegia group and from 32.885±18.771 µg/L to 20.692±6.039 µg/L [12.192; 0.066-24.319; p=0.049] in the ICCF group, thereby resulting in a significant reduction of total hsTnT AUC of 25% and 37% respectively. This therefore demonstrates that an enhanced preconditioning stimulus is able to reduce PMI in patients undergoing cardiac surgery irrespective of the technique of myocardial preservation used.

The vast majority of proof-of-concept studies investigating the effects of RIPC on PMI in cardiac surgery included patients receiving cardioplegia, reflecting the current clinical practice with cardioplegia being the preferred technique of myocardial preservation worldwide: our group (282) was able to demonstrate that RIPC could reduce PMI with a 43% decrease of total cTnT AUC in patients undergoing CABG surgery in a seminal study including a total of 57 subjects: however, 22 patients received cardioplegia and 35 ICCF, which was not an “accurate reflection” of the real world. The same authors (291) then went on to confirm these beneficial effects in 45 patients undergoing CABG with or without valve surgery and receiving cold-blood cardioplegia, with a 42.4% reduction of cTnT AUC. Similar findings resulted in two further studies by Thielmann and colleagues (293, 300, 469) but not by Lomivorotov et

al (297) on patients undergoing CABG surgery and receiving cold crystalloid cardioplegia. Again in the context of cold crystalloid cardioplegia, Wagner and co-workers (295) found that late RIPC only reduced mean cTnI levels 8 hours post-operatively and Kottenberg et al (298) demonstrated that RIPC could reduce PMI in combination with isoflurane but not propofol. Similarly, a strict anaesthetic regime was also used by Karuppasamy and co-workers (294), who found no difference in cTnI, BNP or CK-MB release between intervention groups in a study involving patients undergoing CABG surgery and receiving either cold cardioplegia or ICCF.

Within the context of cold blood cardioplegia, no significant PMI reduction was found (296) in high-risk patients undergoing complex cardiac surgery with a higher mean EuroSCORE than those documented in other clinical studies (7.1 vs 6.6 in RIPC and control groups respectively), in elective or urgent CABG (286) or elective CABG patients receiving opioids and propofol for anaesthesia induction, and isoflurane for anaesthesia maintenance (299).

In summary, both in the context of blood cardioplegia and crystalloid cardioplegia, RCTs have demonstrated positive or negative outcomes in PMI reduction provided by RIPC: a recent survey in the UK and Ireland (30) showed that of the 84.3% of surgeons using cardioplegia (with the remaining 15.7% using ICCF), 83.5% used blood cardioplegia and 16.5% crystalloid cardioplegia: the reasons for this reside in the potential advantage of cold blood cardioplegia of more closely approximating normal physiology, because of the significant amount of oxygen carried by the haemoglobin, the metabolic substrates present in the blood, the physiological buffers and osmotic pressure (470). However, hypothermia induced during surgery might cause a left shift in the oxyhaemoglobin dissociation curve, which can partially counteract these effects, and oxygen supply to the ischaemic myocardium during

intermittent reperfusion might favour the formation of ROS, which could ultimately lead to lethal myocardial IRI (470): clearly these potential complications of the use of blood cardioplegia have not been observed with crystalloid cardioplegia.

Whilst the cardioprotective effects of cardioplegia and ICCF have been demonstrated to be equivalent, a number of studies and systematic reviews (reviewed in (470)) have compared blood cardioplegia (antegrade intermittent or continuous, antegrade/retrograde intermittent or continuous) with crystalloid cardioplegia: a recent meta-analysis (470) concluded that the former is associated with reduced peri-operative MI (17 cases out of 1434 patients versus 32 cases out of 1310 patients (RR=2.30 [1.33, 3.98], p=0.003), although no difference was found in the overall incidence of spontaneous sinus rhythm, mortality within 30 days, new onset of AF and stroke (27). However, no study has so far directly compared the beneficial effects of RIPC on PMI between patients receiving blood or crystalloid cardioplegia. At the tertiary centre where we conducted our study, only blood cardioplegia was used and in chapter 4 we will evaluate further the different types of blood cardioplegia and delivery techniques utlised and their impact on PMI.

Importantly, with regards to the findings in the ICCF group, we could only find one study (468) in the literature in which recruited patients undergoing CABG surgery received ICCF as the only technique of myocardial preservation: however in this RCT, the preconditioning stimulus (IPC) was given invasively by two three minute periods of ischaemia by aortic cross-clamping, each separated by two minutes of reperfusion before the first anastomosis.

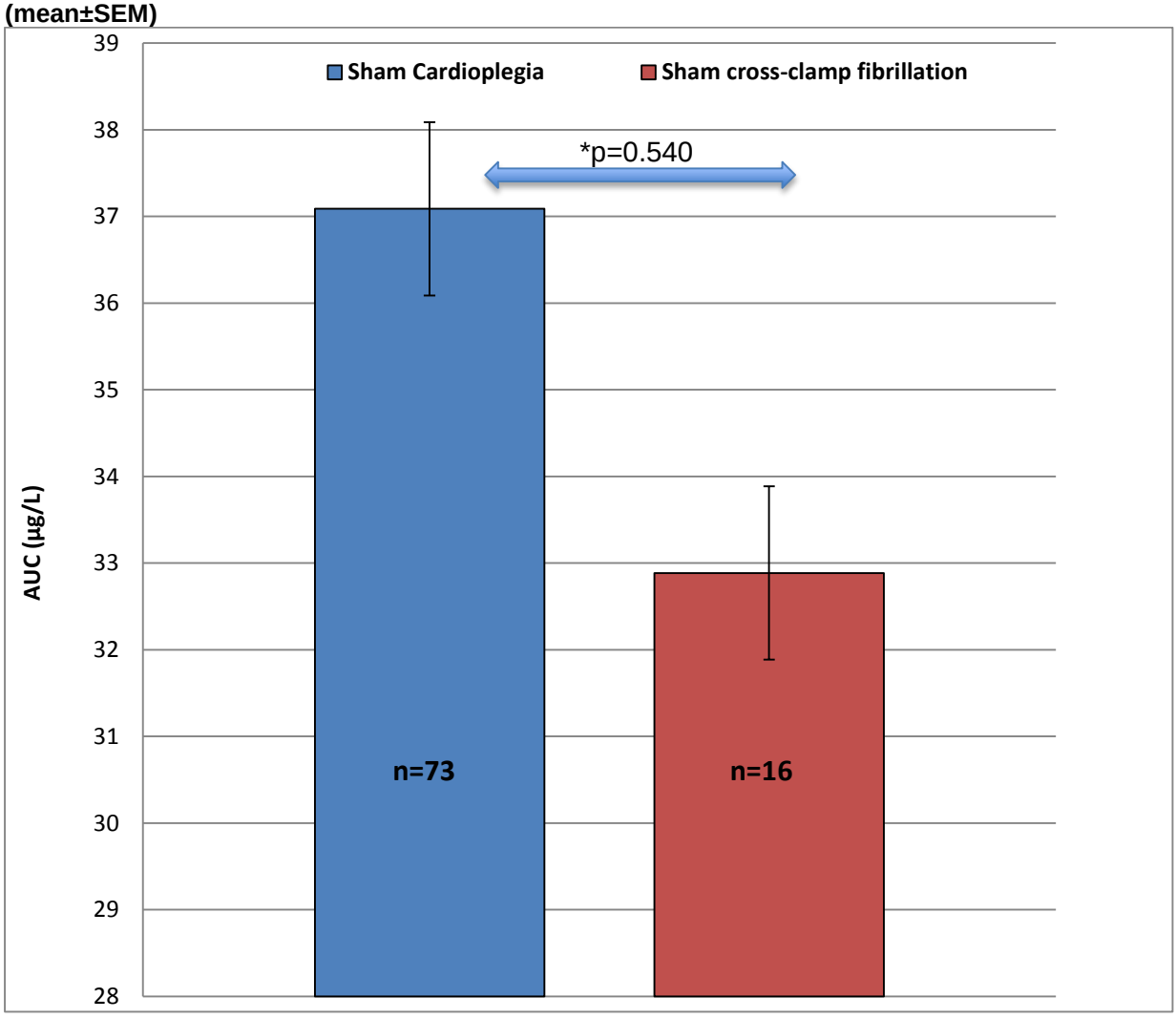
In conclusion, we have showed that our enhanced preconditioning stimulus is able to provide significant cardioprotection in patients undergoing cardiac surgery and receiving either blood cardioplegia or ICCF and therefore irrespective of the technique

of myocardial preservation utilised. As previously described, mechanisms underlying RIPC are yet not entirely understood and therefore it is difficult to explain the potential reason for these findings: certainly, whilst ICCF has not been extensively investigated as the sole technique of myocardial preservation, the role of cardioplegia in RIPC induced cardioprotection in the setting of cardiac surgery remains controversial. However, importantly, whilst the above-mentioned proof-of-concept clinical trials have used a standard RIPC stimulus, in our study we used simultaneous multi-limb IR, which represents a more potent preconditioning stimulus, thereby once again corroborating our hypothesis that the intensity of the RIPC stimulus may play an essential role in the cardioprotective effects induced by this non-invasive strategy.

Another important aspect of our retrospective analysis is that, in accordance with previous studies (31, 89-93), the total AUC of control patients receiving cardioplegia was not statistically significantly different from that of sham subjects undergoing ICCF (respectively 37.089±25.730 µg/L and 32.885±18.771 µg/L [4.204; CI -9.369-17.778; p=0.540] **(Fig. 3.7)**, despite significantly shorter CPB times (100.44±33.12 min versus 77.21±21.98 min [23.22; CI 8.75-37.69; p=0.003]) and cross-clamp times (70.56±24.44 min versus 33.00±7.49 min [37.56; CI 30.46-44.65; p<0.001]) in the ICCF group compared to the cardioplegia group. However, it is important also to confirm that whilst the ICCF group only comprised patients undergoing CABG surgery, the cardioplegia group also consisted of subjects undergoing CABG plus AVR, AVR only, MV surgery and AVR plus MVR with understandably prolonged CPB and cross-clamp times as previously described. Therefore in the next sections we will explain the significance of the impact of CPB and cross-clamp time on PMI in cardiac surgery and we will also describe in more details the most significant differences amongst more homogenous groups of control

patients undergoing CABG surgery alone in both the cardioplegia and ICCF groups in terms of PMI, CPB and cross-clamp times.

Fig. 3.7. Comparison of hsTnT AUC between control patients in the cardioplegia and ICCF group (mean±SEM)



AUC= area under the curve; SEM=standard error of the mean. * Unpaired Student T-Test

3.13.2. Multi-limb RIPC and types of cardiac surgery: effects on cardioprotection in patients undergoing CABG surgery and/or valve surgery

Further to the intriguing findings from the previous sections, where we established that an enhanced preconditioning stimulus is able to reduce PMI in unselected patients undergoing cardiac surgery and irrespective of the type of myocardial preservation, we also wished to establish whether this protective strategy is effective in the context of any type of cardiac surgery, including CABG with or without valve surgery, CABG surgery alone, CABG plus AVR, and valve surgery alone. Therefore we conducted a further series of retrospective analyses using the significant amount of data deriving from our principal study. Within the group comprising CABG surgery alone, we then analysed data deriving from patients receiving cardioplegia as the only technique of myocardial preservation in relation to those undergoing ICCF in order to obtain a direct comparison between more homogenous groups **(Table 3.14)**.

Table. 3.13. Distribution of different types of surgery in control and RIPC groups

Type of Surgery	Control (n=89)	RIPC (n=89)
CABG alone	54 (61%)	57 (64%)
AVR alone	15 (17%)	14 (16%)
CABG+AVR	10 (11%)	9 (10%)
MV Replacement/Repair	9 (10%)	8 (9%)
AVR+MV Replacement/Repair	1 (1%)	1 (1%)

CABG=coronary artery bypass graft; RIPC=Remote ischemic preconditioning; AVR=aortic valve replacement; MV=mitral valve

3.13.2.1. Effects of multi-limb RIPC on cardioprotection in patients undergoing CABG surgery with or without valve surgery

A total of 130 patients underwent CABG surgery with or without valve surgery, of whom 64 received the sham protocol, 66 the preconditioning protocol **(Table 3.15)**. Baseline patients' characteristics were comparable between the two groups **(Table 3.15)** and similarly no statistically significant difference was found in any of the parameters described in **Table 3.16.** In particular no difference was identified with regards to peri-operative risks as indicated by mean EuroSCORE as well as in terms of CPB and cross-clamp times. The different types of operation were again equally distributed in the two groups. Importantly the use of peri-operative GTN was again similar between control and preconditioned subjects, and this will be further discussed in the last section of the current chapter.

Table 3.14. Patient baseline characteristics in patients undergoing CABG surgery with or without valve surgery

Patients	Control (n=64)	RIPC (n=66)	P value
Age	66±9	64±10	0.114
Gender			0.632
Male	53 (82.8%)	57 (86.4%)	
Female	11 (17.2%)	9 (13.6%)	
Ethnicity			0.487
Caucasian	51 (79.7%)	53 (80.3%)	
Asian	9 (14.1%)	12 (18.2%)	
Afro-Caribbean	3 (4.7%)	1 (1.5%)	
Chinese	1 (1.6%)	0 (0%)	
BMI	28.9±5.0	28.0±7.6	0.943
SBP *(mmHg)*	129.9±18.6	128.6±16.6	0.678
DBP *(mmHg)*	70.5±8.7	70.0±8.6	0.760
HR *(bpm)*	68.0±11.7	65.4±9.8	0.167
Smoking History			0.950
Smoker	10 (15.6%)	9 (13.6%)	
Ex-smoker	36 (56.3%)	38 (57.6%)	
Non-smoker	18 (28.1%)	19 (28.8%)	
Family History of IHD	45 (70.3%)	51 (77.3%)	0.427
NYHA Class	2.61±0.9	2.43±0.8	0.229
CCS Class	2.59±1.0	2.65±0.9	0.731
LVEF			0.514
>50%	48 (75.0%)	46 (69.7%)	
30%-50%	14 (21.9%)	15 (22.7%)	
<30%	2 (3.1%)	5 (7.6%)	
Co-morbidities			
Diabetes Mellitus	21 (32.8%)	24 (36.4%)	0.715
Hypertension	54 (84.4%)	50 (75.8%)	0.275
Hypercholesterolemia	56 (87.5%)	54 (81.8%)	0.468
Atrial Fibrillation	7 (11.0%)	4 (6.0%)	0.285
Previous MI	22 (34.4%)	27 (40.9%)	0.473
Previous PCI	10 (15.6%)	10 (15.2%)	1.000
Previous CVA/TIA	6 (9.4%)	4 (6.0%)	0.349
Previous Cardiac Surgery	0 (0.0%)	1 (1.5%)	1.000
Other comorbidities	4 (6.3%)	2 (3.1%)	0.612
Peripheral Arterial Disease	6 (9.4%)	1 (1.5%)	0.060
Drug History			
Aspirin	52 (83.9%)	61 (95.4%)	0.090
Clopidogrel/Prasugrel	23 (37.1%)	22 (34.4%)	0.261
Warfarin	3 (4.8%)	2 (3.1%)	0.594
Beta-blocker	45 (72.6%)	45 (70.3%)	0.845
Calcium Channel Blocker	24 (38.7%)	15 (23.4%)	0.096
Statin	58 (93.5%)	58 (90.6%)	0.494
ACE-I/ARB	43 (69.4%)	42 (65.6%)	0.875
Long acting nitrates	13 (21.0%)	12 (18.8%)	0.589
Antidiabetics			
Insulin	6 (9.7%)	7 (10.9%)	1.000
Biguanide	3 (4.7%)	3 (4.8%)	0.995
Sulphonylurea	9 (14.5%)	6 (9.4%)	0.388
Diuretics	16 (25.8%)	20 (31.3%)	0.672

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA=Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF=left ventricular ejection fraction.

Table 3.15. Details of surgical procedure in patients undergoing CABG surgery with or without valve surgery

Patients	Control (n=64)	RIPC (n=66)	P value
Indication for Surgery			
Angina	44 (68.8%)	40 (60.6%)	0.630
Myocardial Infarction	11 (20.4%)	18 (28.1%)	
Angina and Valve Disease	6 (9.4%)	4 (6.1%)	
Valve disease	2 (3.1%)	2 (3.0%)	
Myocardial infarction and valve disease	1 (1.6%)	2 (3.0%)	
EuroSCORE	3.38±1.86	3.24±2.61	0.740
Additive perioperative risk			0.788
Low (EuroSCORE 0-2)	23 (35.9%)	25 (37.9%)	
Medium (EuroSCORE 3-5)	33 (51.6%)	30 (45.5%)	
High (EuroSCORE >5)	8 (12.5%)	11 (16.7%)	
Bypass-time (min)	93.85±33.47	90.09±30.93	0.716
Cross-clamp time (min)	61.25±27.15	60.14±27.16	0.510
Cardioprotection			0.818
Blood cardioplegia	48 (75.0%)	52 (78.8%)	
Cross-clamp fibrillation	16 (25.0%)	14 (21.2%)	
Number of grafts			0.405
One	4 (6.3%)	5 (7.6%)	
Two	19 (29.7%)	15 (22.7%)	
Three	29 (45.3%)	35 (53.0%)	
Four	12 (18.8%)	11 (16.7%)	
Anesthetic agents			
Induction			
Anti-nicotinic agents			0.763
Rocuronium	46 (76.7%)	56 (88.9%)	
Pancuronium	12 (20.0%)	5 (7.9%)	
Vecuronium	2 (3.3%)	1 (1.6%)	
Midazolam	31 (51.7%)	27 (42.9%)	0.282
Etomidate	7 (11.5%)	6 (9.5%)	0.369
Fentanyl	64 (100%)	63 (100%)	0.776
Propofol	52 (85.2%)	56 (88.9%)	1.000
Maintenance			
Propofol	64 (100%)	63 (100%)	0.600
Volatile Anesthetics			
Isoflurane	56 (91.8%)	60 (95.2%)	1.000
Sevoflurane	5 (8.2%)	3 (4.8%)	0.488
Intra-operative GTN	51 (83.6%)	47 (72.3%)	0.140

RIPC=Remote Ischemic Preconditioning; CABG=Coronary artery bypass graft; AVR=Aortic valve replacement; MVR=Mitral valve replacement; MV=Mitral valve; GTN=glyceryl trinitrate.

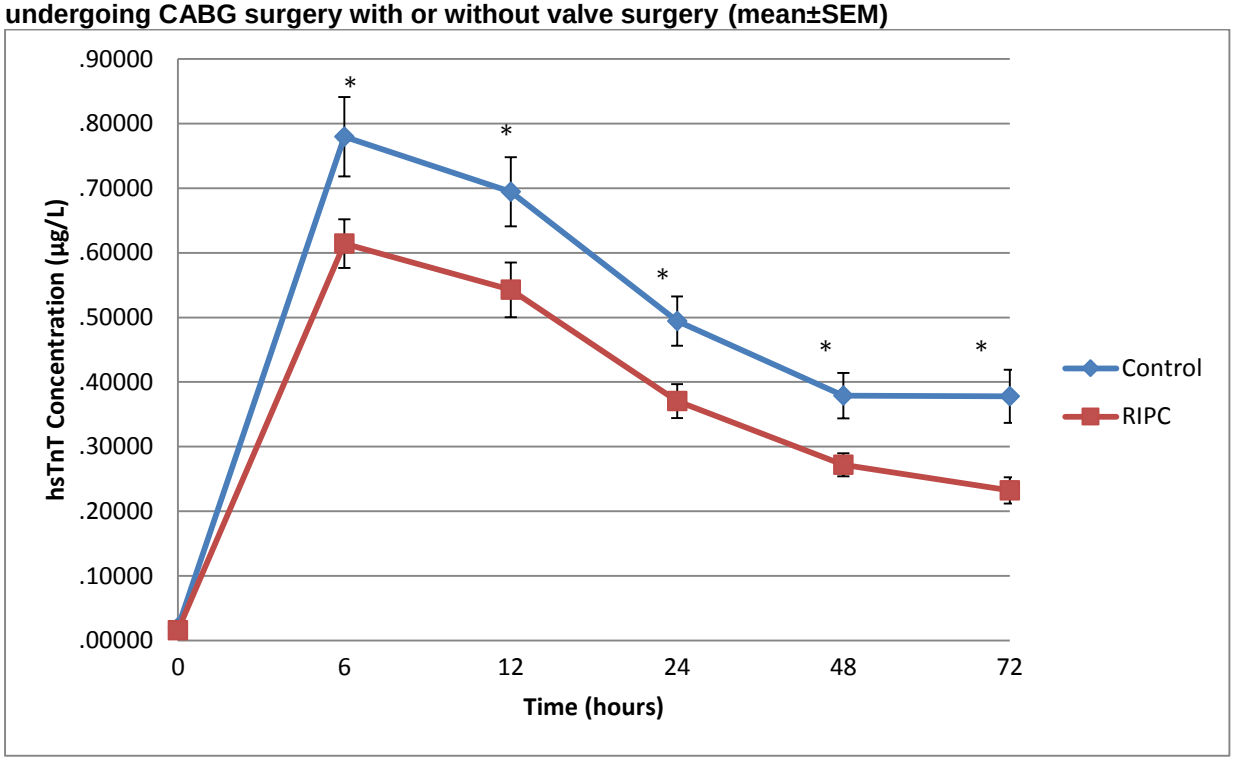
Baseline mean hsTnT was not statistically different between the two intervention groups and, crucially RIPC significantly reduced mean hsTnT at all the studied post-operative time-points **(Table 3.17, Fig. 3.8).** More importantly, total hsTnT release expressed as 72 hours hsTnT AUC was reduced from 33.526±20.164 µg/L in control patients to 24.772±12.640 µg/L in preconditioned subjects [8.753; CI 2.808, 14.688; p=0.004], with a significant reduction of 26% **(Fig. 3.9)**.

Table 3.16. High-sensitivity Troponin-T levels and total AUC post- operatively in patients undergoing CABG surgery with or without valve surgery

Endpoint	Control (n=64) (mean (sd))	RIPC (n=66) (mean (sd))	Difference (95% CI)	P value
hsTnT *(µg/L)*				
Pre-operatively	0.020 (0.018)	0.016 (0.022)	0.004 (-0.003, 0.011)	0.210
6 hours post-operatively	0.780 (0.491)	0.614 (0.306)	0.166 (0.024, 0.307)	0.023
12 hours post-operatively	0.694 (0.428)	0.543 (0.344)	0.152 (0.017, 0.286)	0.027
24 hours post-operatively	0.494 (0.304)	0.370 (0.213)	0.124 (0.033, 0.215)	0.008
48 hours post-operatively	0.379 (0.278)	0.272 (0.144)	0.107 (0.030, 0.184)	0.007
72 hours post-operatively	0.378 (0.325)	0.232 (0.161)	0.147 (0.055, 0.237)	0.002
Total 72 hours AUC	33.526 (20.164)	24.772 (12.640)	8.753 (2.808, 14.688)	0.004

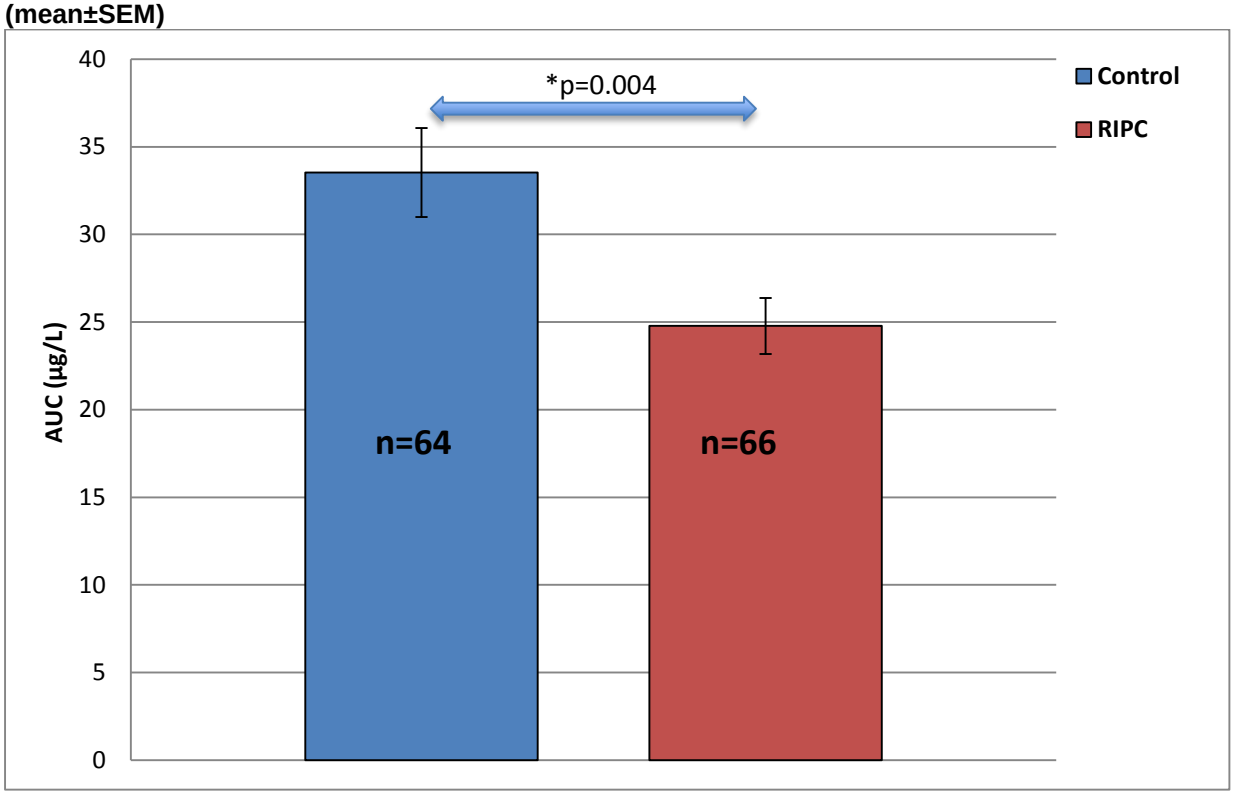
RIPC=Remote ischemic preconditioning; SD=standard deviation; CI=confidence interval; hsTnT=high sensitivity Troponin-T; AUC=area-under-the-curve

Fig. 3.8. High-sensitivity Troponin T at 0, 6, 12, 24, 48 and 72 hours post-operatively in patients undergoing CABG surgery with or without valve surgery (mean±SEM)



RIPC=remote ischaemic preconditioning; hsTnT=high-sensitivity Troponin-T; SEM=standard error of the mean; *p<0.05 (unpaired Student T-Test)

Fig. 3.9. Total hsTnT AUC in patients undergoing CABG surgery with or without valve surgery (mean±SEM)



RIPC=Remote ischaemic preconditioning; AUC=area under the curve; SEM=standard error of the mean. * Unpaired Student T-Test

With regards to our study secondary end-points **(Table 3.18)**, similarly to our previous findings, we found no difference in skeletal muscle injury, inotrope requirement or clinical outcomes at 6 weeks and AKI incidence was reduced by 54% in preconditioned patients, although this did not reach statistical significance. Crucially we observed a significant decrease in new onset AF incidence of 63% (p=0.008) and length of ICU stay of 1 day (p=0.020) albeit not of hospital stay.

Crucially, when we then excluded patients receiving ICCF from our retrospective analysis, we found similar outcomes in the RIPC group compared to control patients **(Table 3.19)**:

1) 24% of total AUC reduction, from 33.74±20.81 μg/L to 25.64±13.52 μg/L [8.11; CI 1.01, 15.21; p=0.026], as well as mean hsTnT release 24 and 72 hours post-operatively;
2) improved urine output over the three days post-surgery from 5830.3±1838.4 mls to 6737.6±1564.8 mls [-907.4; CI -1803.4, -11.3; p=0.047];
3) 60% reduction of new onset AF incidence from 20 to 8 new cases (p=0.004);
4) two days reduction of total ICU stay from a median of 3.0 (IQR: 2.0 - 4.0) days vs 1.0 (IQR: 2.0 – 3.0) days (p=0.033);
5) importantly and uniquely in the present study, a 1.5 day of reduction of total length of hospital stay, from 8.5 (7.0 – 12.0) days vs 7.0 (6.0 – 9.5) days (p=0.050).

Table 3.17. Study secondary end-points in patients undergoing CABG with or without valve surgery*

Endpoint	Control (n=54) (mean (sd))	RIPC (n=57) (mean (sd))	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	34307.86 (21857.37)	36752.39 (26453.21)	-2444.53 (-12580.60, 7691.54)	0.633
Creatinine (mg/ml)				
Pre-operatively	76.72 (14.16)	91.09 (20.10)	4.39 (-3.89, 12.68)	0.296
24 hours post-operatively	94.52 (30.91)	87.47 (26.51)	5.05 (-2.94, 17.03)	0.165
48 hours post-operatively	108.63 (51.25)	91.29 (35.28)	17.34 (2.11, 32.57)	0.026
72 hours post-operatively	104.38 (51.25)	91.29 (26.51)	14.59 (-1.96, 31.13)	0.434
Urine Output (ml)				
24 hours post-operatively	1958.8 (608.7)	2195.02 (659.2)	-236.24 (-470.4, -2.05)	0.048
48 hours post-operatively	2207.3 (964.9)	2354.4 (833.8)	-147.02 (-496.88, 202.84)	0.407
72 hours post-operatively	2003.2 (921.2)	2486.3 (851.2)	-483.05 (-888.15, -77.96)	0.020
Total	5869.4 (1864.9)	6571.1 (1505.2)	-881.76 (-1663.09, 100.44)	0.026
AKI score				
0	51	60		0.295
1	8	4		
2	2	2		
3	3	0		
Acute Kidney Injury	13 (20%)	6 (9%)		0.085
Inotrope score				
Post bypass	5.66 (11.25)	6.38 (15.58)	-0.72 (-5.62, 4.17)	0.770
24 hours post-operatively	10.40 (19.46)	9.21 (16.92)	1.19 (-5.36, 7.74)	0.719
48 hours post-operatively	8.01 (19.79)	5.23 (14.45)	2.78 (-3.44, 9.00)	0.379
72 hours post-operatively	5.58 (18.49)	0.87 (4.16)	4.71 (-0.29, 9.72)	0.065
Total	29.96 (57.97)	21.69 (43.03)	8.27 (-10.37, 26.91)	0.381
New onset AF	19 30%)	7 (11%)		0.008
Length of ICU stay (days)	3.0 (2.0-4.5)**	2.0 (1.0-4.0)**		0.020***
Length of hospital stay (days)	8.0 (6.5-11.5)**	7.5 (6.0-9.0)**		0.075***
Clinical outcomes at six weeks				
Death	3	0		0.118
Myocardial infarction	1	0		0.593
Stroke	0	0		1.000
Revascularization	0	0		1.000

Table 3.18. Study end-points in patients undergoing CABG with or without valve surgery with cardioplegia *

Endpoint	Control (n=48) (mean (sd))	RIPC (n=52) (mean (sd))	Difference (95% CI)	P value
hsTnT *(µg/L)*				
Pre-operatively	0.020 (0.017)	0.017 (0.023)	0.003 (-0.006, 0.011)	0.513
6 hours post-operatively	0.808 (0.549)	0.642 (0.325)	0.166 (-0.016, 0.348)	0.073
12 hours post-operatively	0.716 (0.481)	0.566 (0.374)	0.150 (-0.022, 0.322)	0.087
24 hours post-operatively	0.501 (0.307)	0.387 (0.233)	0.114 (0.005, 0.223)	0.041
48 hours post-operatively	0.368 (0.257)	0.283 (0.155)	0.085 (-0.001, 0.171)	0.052
72 hours post-operatively	0.377 (0.345)	0.241 (0.174)	0.135 (0.024, 0.247)	0.018
Total 72 hours AUC	33.74 (20.81)	25.64 (13.52)	8.11 (1.01, 15.21)	0.026
CK (*µg/L*)				
Total AUC	31481.3 (14549.4)	36045.2 (23211.8)	-4563.8 (-13476.1, 4348.4)	0.311
Creatinine (mg/ml)				
Pre-operatively	92.1 (19.9)	84.6 (25.2)	7.45 (-1.6, 16.5)	0.106
24 hours post-operatively	90.2 (17.5)	86.9 (26.7)	3.3 (-7.1, 13.6)	0.530
48 hours post-operatively	101.3 (36.0)	89.1 (34.4)	12.2 (-1.8, 26.2)	0.086
72 hours post-operatively	99.4 (52.1)	88.1 (33.3)	11.3 (-5.9, 28.5)	0.198
Urine Output (ml)				
24 hours post-operatively	1964.0 (545.3)	2171.1 (567.3)	-207.1 (-440.9, 26.9)	0.082
48 hours post-operatively	2252.0 (975.6)	2297.4 (781.1)	-45.4 (-429.5, 338.7)	0.815
72 hours post-operatively	1873.9 (828.2)	2472.9 (919.9)	-599.0 (-1060.6, -137.4)	0.012
Total	5830.3 (1838.4)	6737.6 (1564.8)	-907.4 (-1803.4, -11.3)	0.047
AKI score				
0	40	47		0.114
1	6	3		
2	0	2		
3	2	0		
Acute Kidney Injury	8	5		0.377
Inotrope score				
Post bypass	6.2 (11.1)	7.5 (17.2)	-1.3 (-7.3, 4.6)	0.655
24 hours post-operatively	10.5 (18.5)	9.7 (17.7)	0.8 (-6.7, 8.2)	0.839
48 hours post-operatively	5.8 (12.3)	5.8 (15.5)	-0.1 (-5.9, 5.8)	0.980
72 hours post-operatively	2.9 (9.4)	0.4 (1.8)	2.6 (-0.4, 5.5)	0.084
Total	25.9 (40.5)	23.5 (45.4)	2.4 (-15.5, 20.2)	0.793
New onset AF	20	8		0.004
Length of ICU stay (days)	3.0 (2.0-4.0)**	1.0 (2.0–3.0)**		0.033***
Length of hospital stay (days)	8.5 (7.0–12.0)**	7.0 (6.0–9.5)**		0.050***

Clinical outcomes at six weeks				
Death	3	0		0.240
Myocardial infarction	1	0		0.561
Stroke	0	0		1.000
Revascularization	0	0		1.000

*List of abbreviations
RIPC=Remote ischemic preconditioning; CK=Creatinine Kinase; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve
*P-value for test of interaction between RIC and GTN given; **Results shown as median (inter-quartile range); *** P-value for Mann-Whitney-Wilcoxon test

3.13.2.2. Effects of multi-limb RIPC on cardioprotection in patients undergoing CABG surgery alone

From the cohort of patients recruited into the main study, 111 subjects underwent CABG surgery alone, of which 54 were randomised to control and 57 to RIPC. Again patients' baseline characteristics were essentially similar both in control and preconditioned patients, with the only exception of PAD, which was significantly more frequent in the sham group compared to the RIPC group with 6 vs 1 patients respectively with known PAD (p=0.043) **(Table 3.19)**. With regards to parameters related to surgery, as observed in the main analysis, we found that the only statistically significant difference was the administration of intra-operative GTN, with 46 control patients versus 41 RIPC patients receiving iv nitrates during the operation (p=0.045) **(Table 3.20)**.

Table 3.19. Baseline characteristics in patients undergoing CABG surgery alone

Patients	Control (n=54) (mean (SD))	RIPC (n=57) (mean (SD))	P value
Age	66±9	64±10	0.198
Gender			0.453
Male	43 (79.6%)	49 (86.0%)	
Female	11 (20.4%)	8 (14.0%)	
Ethnicity			0.520
Caucasian	43 (79.6%)	48 (84.2%)	
Asian	7 (13.0%)	8 (14.0%)	
Afro-Caribbean	3 (5.6%)	1 (1.8%)	
Chinese	1 (1.9%)	0 (0%)	
BMI	28.4±4.9	28.4±7.9	0.453
SBP *(mmHg)*	130.2±20	127.4±16.2	0.454
DBP *(mmHg)*	70.8±9.2	69.4±8.9	0.388
HR *(bpm)*			
	67.1±11.3	64.4±9.6	0.188
Smoking History			0.787
Smoker	9 (16.7%)	8 (14.0%)	
Ex-smoker	31 (57.4%)	31 (54.4%)	
Non-smoker	14 (25.9%)	18 (31.6%)	
Family History of IHD	39 (72.2%)	42 (73.7%)	0.862
NYHA Class	2.45±0.8	2.41±0.8	0.784
CCS Class	2.59±1.0	2.70±0.9	0.549
LVEF			0.652
>50%	38 (70.4%)	41 (71.9%)	
30%-50%	14 (25.9%)	12 (21.1%)	
<30%	2 (3.7%)	4 (7.0%)	
Co-morbidities			
Diabetes Mellitus	17 (31.5%)	19 (33.3%)	0.835
Hypertension	45 (83.3%)	42 (73.7%)	0.217
Hypercholesterolemia	48 (88.9%)	46 (80.7%)	0.231
Atrial Fibrillation	5 (9.3%)	4 (7.0%)	0.584
Previous MI	22 (40.7%)	22 (38.6%)	0.817
Previous PCI	9 (20.8%)	9 (15.8%)	0.900
Previous CVA/TIA	6 (16.7%)	3 (5.3%)	0.355
Previous Cardiac Surgery	0 (0.0%)	1 (1.8%)	0.328
Other comorbidities	4 (7.5%)	2 (3.6%)	0.249
Peripheral Arterial Disease	6 (11.1%)	1 (1.8%)	0.043
Drug History			
Aspirin	46 (88.4%)	53 (96.4%)	0.254
Clopidogrel/Prasugrel	21 (40.3%)	19 (34.5%)	0.255
Warfarin	3 (5.7%)	2 (3.6%)	0.585
Beta-blocker	41 (78.8%)	40 (72.7%)	0.461
Calcium Channel Blocker	21 (40.3%)	12 (21.8%)	0.066
Statin	50 (94.2%)	50 (90.9%)	0.455
ACE-I/ARB	36 (69.2%)	38 (69.1%)	0.964
Long acting nitrates	13 (25.0%)	11 (20.0%)	0.532
Antidiabetics			
Insulin	6 (11.5%)	7 (12.7%)	0.851
Biguanide	12 (23.1%)	13 (23.7%)	0.991
Sulphonylurea	7 (13.5%)	4 (7.3%)	0.324
Diuretics	13 (25.0%)	15 (27.3%)	0.761

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA=Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF=left ventricular ejection fraction.

Table 3.20. Details of surgical procedure in patients undergoing CABG surgery alone

Patients	Control (n=54) (mean (SD))	RIPC (n=57) (mean (SD))	P value
Indication for Surgery			0.376
Angina	43 (79.6%)	40 (70.2%)	
Myocardial Infarction	11 (20.4%)	16 (28.1%)	
Angina and Valve Disease	0 (0.0%)	1 (1.8%)	
EuroSCORE	3.17±1.83	2.84±2.39	0.425
Additive perioperative risk			0.788
Low (EuroSCORE 0-2)	22 (40.7%)	25 (43.9%)	
Medium (EuroSCORE 3-5)	26 (48.1%)	24 (42.1%)	
High (EuroSCORE >5)	6 (11.1%)	8 (14.0%)	
Bypass-time (min)	86.77±29.07	85.84±21.81	0.695
Cross-clamp time (min)	55.06±23.3	54.84±19.29	0.956
Cardioprotection			
Blood cardioplegia	18 (75.0%)	23 (82.1%)	
Cross-clamp fibrillation	6 (25.0%)	5 (17.9%)	
Number of grafts			0.985
One	1 (1.9%)	1 (1.8%)	
Two	13 (24.1%)	14 (24.6%)	
Three	28 (51.9%)	31 (54.4%)	
Four	12 (22.2%)	11 (19.3%)	
Anesthetic agents			
Induction			
Anti-nicotinic agents			0.335
Rocuronium	42 (84.0%)	49 (90.7	
Pancuronium	6 (12.0%)	3 (5.6%)	
Vecuronium	2 (4.0%)	2 (3.7%)	
Midazolam	25 (47.9%)	22 (40.7%)	0.343
Etomidate	5 (9.8%)	5 (9.3%)	0.924
Fentanyl	54 (100%)	57 (100%)	1.000
Propofol	49 (90.2%)	52 (88.9%)	0.827
Maintenance			
Propofol	54 (100%)	57 (100%)	1.000
Volatile Anesthetics			0.639
Isoflurane	47 (92.2%)	51 (94.4%)	
Sevoflurane	4 (7.8%)	3 (5.6%)	
Intra-operative GTN	46 (88.5%)	41 (73.2%)	0.045

RIPC= Remote Ischemic Preconditioning; GTN=glyceryl trinitrate

As found in group and subgroup analyses so far performed, baseline hsTnT levels were similar in the two groups and importantly mean hsTnT concentrations were significantly lower in RIPC patients at all the specified time-points **(Table 3.21, Fig.2.11)**.

Table 3.21. High-sensitivity Troponin-T release at the specified time points in patients undergoing CABG surgery alone

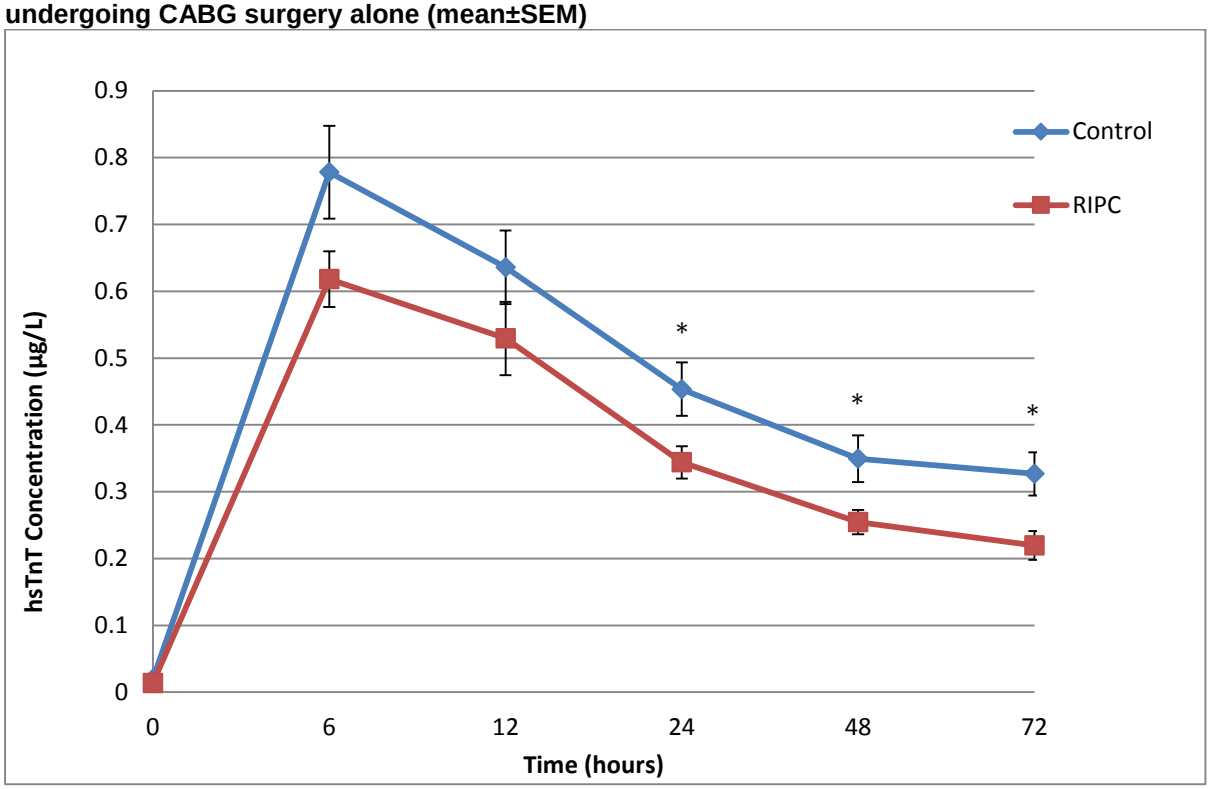
Endpoint	Control (n=54) (mean (sd))	RIPC (n=57) (mean (sd))	Difference (95% CI)	P value
hsTnT *(µg/L)*				
Pre-operatively	0.019 (0.019)	0.013 (0.020)	0.003 (-0.001, 0.014)	0.091
6 hours post-operatively	0.778 (0.509)	0.618 (0.314)	0.160 (-0.001, 0.321)	0.051
12 hours post-operatively	0.636 (0.404)	0.529 (0.353)	0.106 (-0.362, 0.249)	0.142
24 hours post-operatively	0.453 (0.294)	0.344 (0.182)	0.109 (0.168, 0.202)	0.021
48 hours post-operatively	0.349 (0.182)	0.254 (0.135)	0.095 (0.016, 0.173)	0.018
72 hours post-operatively	0.327 (0.235)	0.219 (0.157)	0.107 (0.030, 0.184)	0.007
Total 72 hours AUC	30.753 (18.948)	23.609 (12.004)	7.14 (1.076, 13.21)	0.022

RIPC=Remote ischemic preconditioning; SD=standard deviation; CI=confidence interval; hsTnT=high-sensitivity Troponin-T

More interestingly total hsTnT in preconditioned patients was reduced from 30.753±18.949 µg/L to 23.609±12.004 µg/L [7.14; CI (1.076, -13.21; p=0.022], which corresponded to a statistically significant reduction of 23%, thereby demonstrating that transient simultaneous multi-limb IR can reduce PMI in patients undergoing CABG surgery alone **(Table 3.21, Fig.2.12)**.

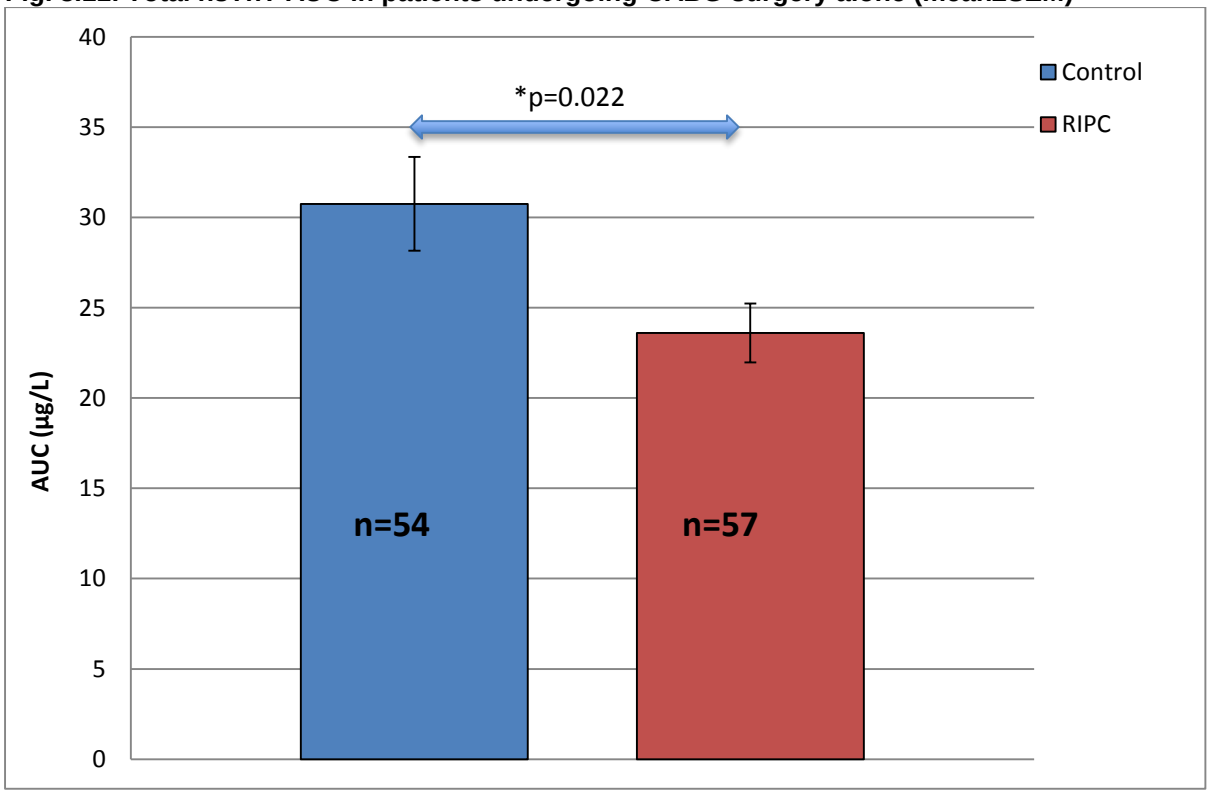
With regards to the study secondary end-points **(Table 3.22)**, we were able to confirm a statistically significant 60% reduction of the onset of new post-operative AF, with 15 new cases in the control group and 6 new cases amongst preconditioned patients (p=0.028) as well as a 55% reduction of AKI incidence, although again this did not reach statistical significance (p=0.107). We also observed 3 deaths and 1 MI in the sham group with no events in the preconditioned group at the six weeks follow-up (p=0.108 and 0.575 respectively).

Fig. 3.10. High-sensitivity Troponin T at 0, 6, 12, 24, 48 and 72 hours post-surgery in patients undergoing CABG surgery alone (mean±SEM)



RIPC=remote ischaemic preconditioning; hsTnT= high-sensitivity Troponin-T; SEM=standard error of the mean; *p<0.05 (unpaired Student T-Test)

Fig. 3.11. Total hsTnT AUC in patients undergoing CABG surgery alone (mean±SEM)



RIPC=remote ischaemic preconditioning; AUC=area-under-the-curve; SEM=standard error of the mean. * Unpaired Student T-Test

Table 3.22. Summary of major secondary endpoints in patients undergoing CABG surgery alone

Endpoint	Control (n=54) (mean (sd))	RIPC (n=57) (mean (sd))	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	34387.11 (23849.96)	35267.12 (27496.90)	-880.01 (-12749.70, 10989.69)	0.883
Creatinine (mg/ml)				
Pre-operatively	88.98 (19.43)	86.14 (25.21)	2.84 (-5.7, 11.3)	0.509
24 hours post-operatively	93.50 (32.82)	85.68 (23.24)	7.82 (-2.89, 18.54)	0.151
48 hours post-operatively	107.09 (54.21)	89.93 (30.38)	17.16 (0.45, 33.88)	0.044
72 hours post-operatively	99.87 (49.88)	89.16 (33.32)	10.71 (-5.17, 26.59)	0.184
Urine Output (ml)				
24 hours post-operatively	1966.0 (649.7)	2195.06 (680.0)	-229.1 (-498.3, 40.2)	0.095
48 hours post-operatively	2206.0 (939.6)	2291.9 (803.5)	-85.70 (-475.1, 163.6)	0.662
72 hours post-operatively	2122.9 (939.6)	2399.2 (792.3)	-276.3 (-716.1, -35.44)	0.214
Total	6002.6 (2012.3)	6550.6 (1375.8)	-547.9 (-1411.7, 315.8)	0.209
AKI score				
0	43	52		0.295
1	7	4		
2	2	1		
3	2	0		
Acute Kidney Injury	11	5		0.107
Inotrope score				
Post bypass	5.61 (12.57)	4.45 (9.63)	1.16 (-5.279, 7.592)	0.719
24 hours post-operatively	9.04 (18.90)	8.38 (13.69)	0.661 (-8.808, 10.131)	0.893
48 hours post-operatively	10.52 (22.53)	4.80 (12.87)	5.722 (-4.661, 16.105)	0.273
72 hours post-operatively	8.55 (20.99)	0.57 (2.45)	7.981 (-0.196, 16.157)	0.098
Total	30.55 (62.65)	19.34 (41.19)	11.21 (-9.48, 31.89)	0.285
New onset AF	15	6		0.028
Length of ICU stay (days)	2.0 (2.0–4.0)**	2.0 (1.0-3.0)**		0.567***
Length of hospital stay (days)	8.0 (6.0-11.0)**	7.0 (6.0-9.0)**		0.784***
Clinical outcomes at six weeks				
Death	3	0		0.108
Myocardial infarction	1	0		0.575
Stroke	0	0		1.000
Revascularization	0	0		1.000

RIPC=Remote ischemic preconditioning; CK=Creatinine Kinase; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve

**Results shown as median (inter-quartile range); *** P-value for Mann-Whitney-Wilcoxon test

3.13.2.3. Effects of multi-limb RIPC on cardioprotection in patients undergoing CABG surgery only with cardioplegia

We then proceeded to analyse data from the 81 patients undergoing CABG surgery alone and receiving blood cardioplegia as the only technique of cardioprotection. Of these, 38 patients received the sham protocol and 43 the RIPC protocol. We found no significant difference in baseline characteristics between the two intervention groups **(Table 3.23)**. Similarly, surgical parameters were not different and in particular, EuroSCORE, CPB and cross-clamp times were comparable between sham and preconditioned patients **(Table 3.24)**. Interestingly, the use of intra-operative GTN was not statistically different between groups.

Table 3.23. Patient baseline characteristics in patients undergoing CABG surgery with cardioplegia

Patients	Control (n=38) (mean (SD))	RIPC (n=43) (mean (SD))	P value
Age	68±8	65±9	0.208
Gender			0.983
Male	31 (81.6%)	35 (81.4%)	
Female	7 (18.4%)	8 (18.6%)	
Ethnicity			0.626
Caucasian	30 (78.9%)	35 (81.4%)	
Asian	5 (13.2%)	7 (16.3%)	
Afro-Caribbean	2 (5.3%)	1 (2.3%)	
Chinese	1 (2.6%)	0 (0%)	
BMI	27.7±4.7	29.4±8.9	0.286
SBP *(mmHg)*	130.6±20.6	127.8±17.9	0.514
DBP *(mmHg)*	69.9±8.6	69.3±9.4	0.752
HR *(bpm)*	66.5±11.7	63.9±9.4	0.978
Smoking History			0.976
Smoker	5 (13.2%)	6 (14.0%)	
Ex-smoker	23 (60.5%)	25 (58.1%)	
Non-smoker	10 (26.3%)	12 (27.9%)	
Family History of IHD	26 (68.4%)	32 (74.4%)	0.625
NYHA Class	2.51±0.77	2.46±0.81	0.781
CCS Class	2.65±1.1	2.66±0.97	0.966
LVEF			0.513
>50%	26 (68.4%)	31 (72.1%)	
30%-50%	11 (28.9%)	9 (20.9%)	
<30%	1 (2.6%)	3 (7.0%)	
Co-morbidities			
Hypertension	32 (84.2%)	33 (76.7%)	0.577
Hypercholesterolemia	33 (86.8%)	34 (79.1%)	0.394
Diabetes Mellitus	11 (28.9%)	14 (32.6%)	0.812
Atrial Fibrillation	4 (10.5%)	1 (2.3%)	0.286
Previous MI	19 (50.0%)	17 (39.5%)	0.377
Previous PCI	8 (21.1%)	6 (14.0%)	0.557
Previous CVA/TIA	6 (15.8%)	3 (6.9%)	0.166
Previous Cardiac Surgery	0 (0.0%)	1 (2.3%)	0.344
Other comorbidities	2 (7.4%)	1 (3.4%)	0.213
Peripheral Arterial Disease	5 (13.2%)	1 (2.3%)	0.094
Drug History			
	34 (89.5%)	40 (95.2%)	0.607
Aspirin	18 (48.4%)	15 (35.7%)	0.221
Clopidogrel/Prasugrel	3 (7.9%)	1 (2.4%)	0.447
Warfarin	30 (78.9%)	28 (66.7%)	0.316
Beta-blocker	16 (42.1%)	10 (23.8%)	0.051
Calcium Channel Blocker	36 (98.0%)	38 (96.0%)	0.281
Statin	26 (68.4%)	29 (69.0%)	0.474
ACE-I/ARB	10 (26.3%)	9 (21.4%)	0.640
Long acting nitrates			
Anti-diabetics	4 (10.5%)	4 (9.5%)	0.881
Insulin	5 (13.2%)	4 (9.5%)	0.489
Sulphonylurea	2 (5.3%)	3 (7.1%)	0.935
Biguanide	9 (23.7%)	10 (23.8%)	0.662
Diuretics			

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA=Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF=left ventricular ejection fraction.

Table 3.24. Details of surgical procedure in patients undergoing CABG surgery with cardioplegia

Patients	Control (n=38) (mean (SD))	RIPC (n=43) (mean (SD))	P value
Indication for Surgery			0.300
Angina	30 (78.9%)	28 (65.1%)	
Myocardial Infarction	8 (21.1%)	9 (32.6%)	
EuroSCORE	3.37±1.87	3.14±2.34	0.636
Additive perioperative risk			0.912
Low (EuroSCORE 0-2)	14 (36.8%)	16 (37.2%)	
Medium (EuroSCORE 3-5)	19 (50.0%)	20 (46.5%)	
High (EuroSCORE >5)	5 (13.2%)	7 (16.3%)	
Bypass-time (min)	90.29±30.79	87.30±23.20	0.621
Cross-clamp time (min)	62.61±21.86	61.21±17.66	0.752
Graft			0.810
One			
Two	1 (3.8%)	1 (3.6%)	
Three	6 (22.2%)	9 (31.0%)	
Four	14 (51.9%)	15 (51.7%)	
	6 (22.2%)	4 (13.8%)	
Anesthetic agents			
Induction			
Anti-nicotinic agents			0.307
Rocuronium	30 (81.1%)	38 (92.7%)	
Pancuronium	5 (13.5%)	1 (4.9%)	
Vecuronium	2 (5.4%)	1 (2.4%)	
Midazolam	18 (48.6%)	17 (41.5%)	0.649
Etomidate	3 (8.1%)	5 (12.2%)	0.715
Fentanyl	38 (100%)	43 (100%)	1.000
Propofol	34 (91.9%)	35 (85.4%)	0.487
Maintenance			
Propofol	38 (100%)	43 (100%)	1.000
Volatile Anesthetics			0.341
Isoflurane	34 (91.9%)	40 (97.6%)	
Sevoflurane	3 (8.1%)	1 (2.4%)	
Intra-operative GTN	32 (88.9%)	29 (69.0%)	0.053

RIPC= Remote Ischemic Preconditioning; GTN=glyceryl trinitrate

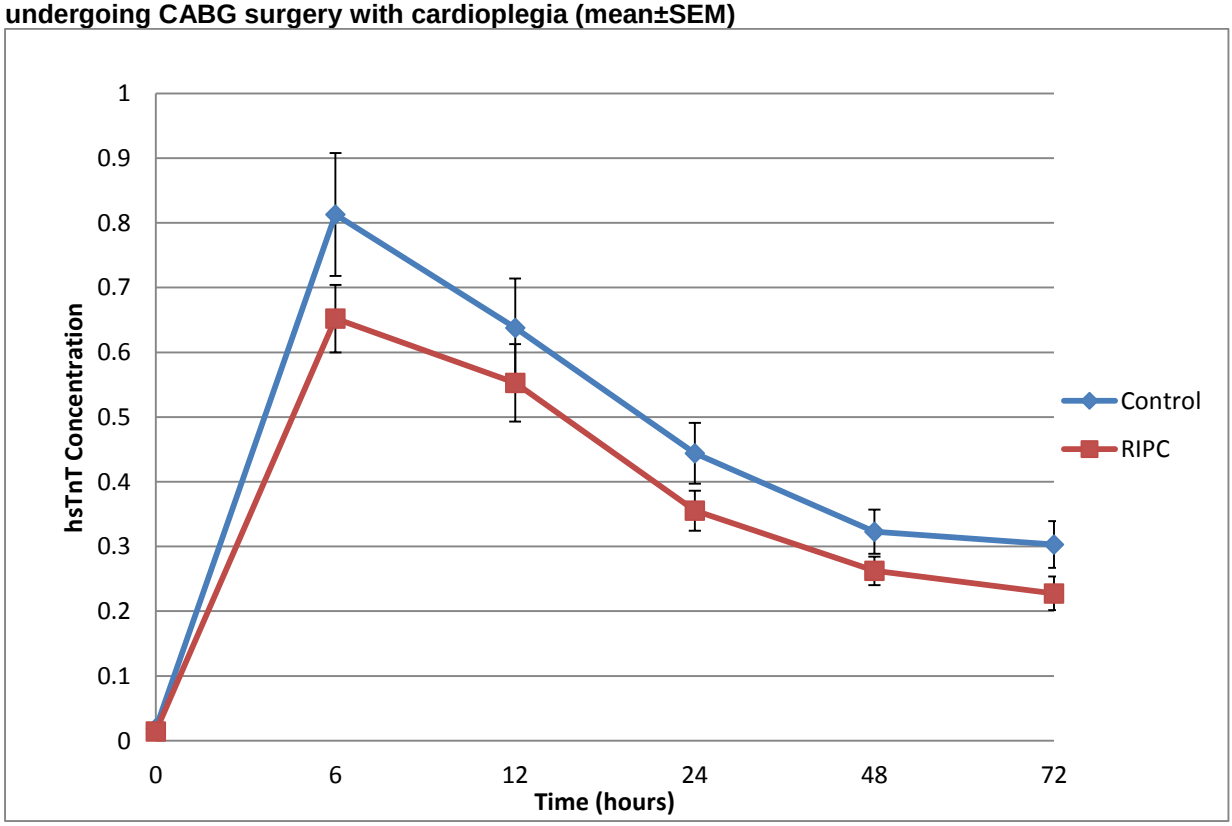
Crucially and in contrast with what we have found so far, although mean hsTnT was lower in the RIPC at each of six the measured time-points, this did not reach statistical significance **(Table 3.25)**. More importantly, we found a non-statistically significant reduction of total hsTnT AUC of 18% only, from 29.832±19.206 μg/L in the control group to 24.355±13.052 μg/L in the RIPC group [5.477 CI -1.985-12.938; p=0.147] **(Table 3.25, Fig. 3.13)**.

Table 3.25. High-sensitivity Troponin-T release in patients undergoing CABG surgery with cardioplegia

Endpoint	Control (n=38) (mean [SD])	RIPC (n=43) (mean [SD])	Difference (95% CI)	P value
hsTnT *(µg/L)*				
Pre-operatively	0.019 (0.019)	0.014 (0.023)	0.005 (-0.004, 0.015)	0.285
6 hours post-operatively	0.813 (0.588)	0.653 (0.339)	0.159 (-0.572, 0.377)	0.146
12 hours post-operatively	0.638 (0.468)	0.553 (0.391)	0.085 (-0.107, 0.278)	0.381
24 hours post-operatively	0.444 (0.292)	0.355 (0.201)	0.056 (-0.024, 0.202)	0.089
48 hours post-operatively	0.323 (0.208)	0.262 (0.147)	0.061 (-0.021, 0.142)	0.132
72 hours post-operatively	0.303 (0.218)	0.228 (0.173)	0.076 (-0.011, 0.163)	0.087
Total 72 hours AUC	29.832 (19.206)	24.355 (13.052)	5.477 (-1.985, 12.938)	0.147

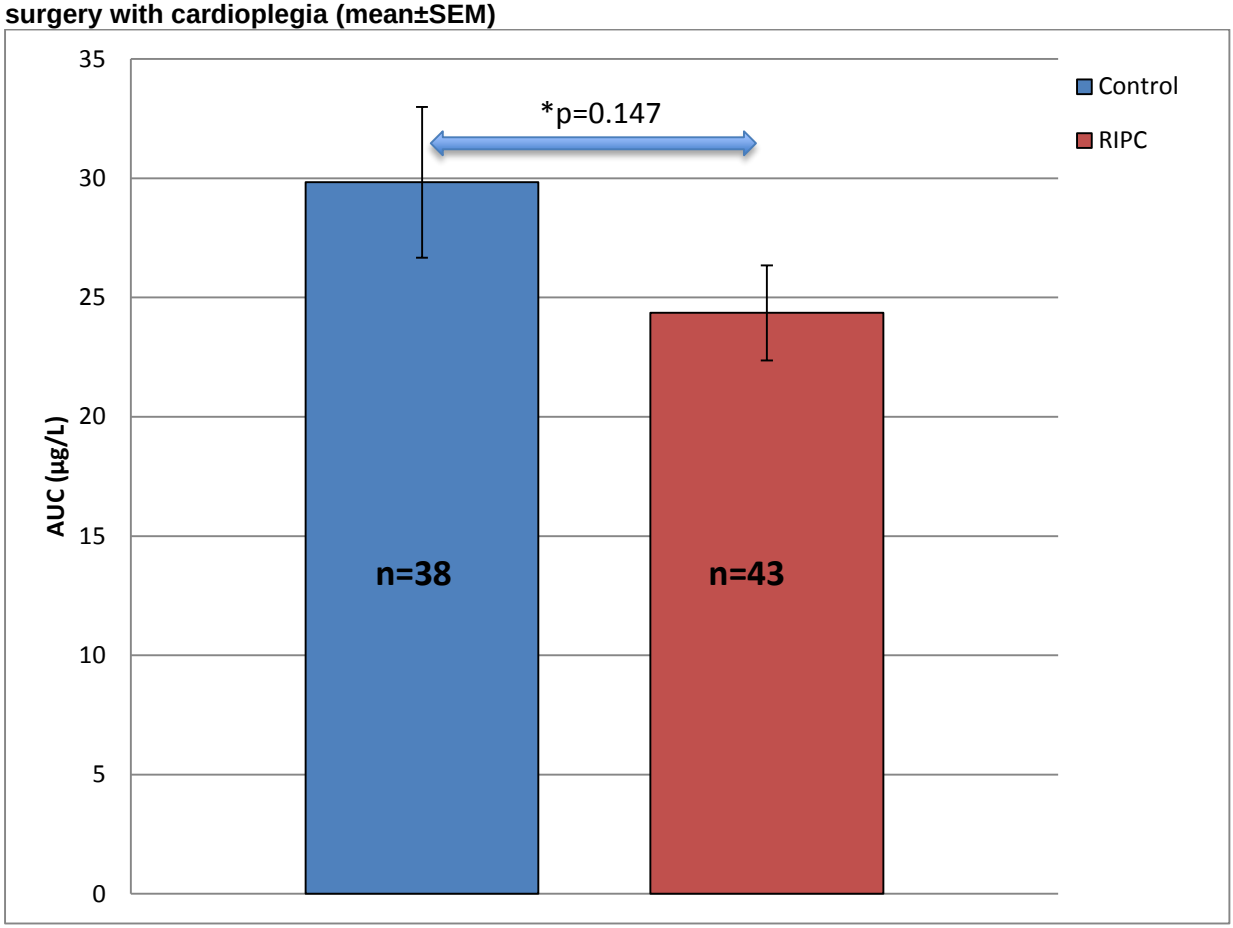
RIPC=Remote ischemic preconditioning; SD= standard deviation; CI= confidence interval; hsTnT=high sensitivity Troponin-T

Fig. 3.12. High-sensitivity Troponin-T at 0, 6, 12, 24, 48 and 72 hours post-operatively in patients undergoing CABG surgery with cardioplegia (mean±SEM)



RIPC=remote ischaemic preconditioning; hsTnT= high-sensitivity Troponin-T; SEM=standard error of the mean. *Unpaired Student T-Test

Fig. 3.13. Total Area under the Curve of high-sensitivity Troponin-T in patients undergoing CABG surgery with cardioplegia (mean±SEM)



RIPC=remote ischaemic preconditioning; AUC=area under the curve; SEM=standard error of the mean.
* Unpaired Student T-Test

Similarly, secondary outcomes were comparable in the two groups **(Table 3.26)**: in particular, even though we observed a 50% reduction of the onset of new AF post-operatively from 10 to 5 new cases, this was not statistically significant (p=0.150)

Table 3.26. Summary of study endpoints in patients undergoing CABG surgery with cardioplegia

Endpoint	Control (n=38) (mean [SD])	RIPC (n=43) (mean [SD])	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	30772.82 (15660.18)	34087.77 (23750.26)	-3314.94 (-13783.98, 7154.09)	0.529
Creatinine (mg/ml)				
Pre-operatively	89.3 (19.0)	83.4 (21.9)	5.9 (-3.6, 15.0)	0.204
24 hours post-operatively	87.6 (26.5)	84.4 (21.9)	3.3 (-7.5, 13.9)	0.547
48 hours post-operatively	97.2 (36.4)	86.8 (26.9)	10.4 (-3.7, 24.4)	0.146
72 hours post-operatively	91.7 (38.4)	86.9 (26.4)	4.7 (-10.1, 19.5)	0.516
Urine Output (ml)				
24 hours post-operatively	1975.8 (589.0)	2165.6 (576.3)	-189.8 (-466.7, 87.2)	0.176
48 hours post-operatively	2265.7 (1041.7)	2203.7 (715.0)	62.6 (-373.9, 499.0)	0.776
72 hours post-operatively	1989.6 (843.5)	2346.1 (854.5)	-356.5 (-875.4, 162.5)	0.173
Total	6001.2 (2039.6)	6450.2 (1388.1)	-449.0 (-1542.0, 644.0)	0.392
AKI score				
0	32 (84.2%)	39 (90.7%)		0.408
1	5 (13.2%)	3 (7.0%)		
2	0 (0.0%)	1 (2.3%)		
3	1 (2.6%)	0 (0.0%)		
Acute Kidney Injury	6 (15.8%)	4 (9.3%)		0.503
Inotrope score				
Post bypass	6.35 (12.11)	6.97 (17.26)	-0.63 (-7.56, 6.30)	0.857
24 hours post-operatively	9.72 (18.68)	8.59 (16.85)	1.13 (-7.05, 9.32)	0.783
48 hours post-operatively	7.11 (13.72)	4.72 (14.61)	2.38 (-4.25, 9.02)	0.476
72 hours post-operatively	1.91 (4.79)	0.52 (1.99)	1.39 (-0.25, 3.04)	0.125
Total	25.46 (43.73)	20.79 (43.79)	4.66 (-15.75, 25.08)	0.650
New onset AF	10 (26.3%)	5 (11.6%)		0.150
Length of ICU stay (days)	2.0 (2.0-4.0)**	2.0 (1.0 – 3.0)**		0.188***
Length of hospital stay (days)	8.0 (7.0-11.0)**	7.0 (6.0 – 9.0)**		0.102***
Clinical outcomes at six weeks				
Death	3 (10.3%)	0 (0.0%)		0.107
Myocardial infarction	1 (2.6%)	0 (0.0%)		0.528
Stroke	0 (0.0%)	0 (0.0%)		1.000
Revascularization	0 (0.0%)	0 (0.0%)		1.000

RIPC=Remote ischemic preconditioning; CK =Creatinine Kinase; AKI =Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve

**Results shown as median (inter-quartile range); *** P-value for Mann-Whitney-Wilcoxon test

3.13.2.4. Discussion

In these retrospective analyses we have been able to demonstrate that our enhanced preconditioning stimulus can reduce PMI, new AF incidence and total ICU stay in patients undergoing CABG surgery with or without valve surgery. The same results were obtained when in the analysis we only included subjects undergoing CABG with or without valve surgery and receiving cardioplegia, although we found no statistically significant difference of any of the study end-points in the context of CABG surgery alone with cardioplegia or CABG plus AVR surgery. Crucially and uniquely in this study and from the current literature, we found a reduction of 1.5 days of total hospital stay duration although with a weaker statistical significance ($p=0.050$) in RIPC patients undergoing CABG with or without valve surgery with cardioplegia only.

In the first instance, our findings confirm the positive effects of RIPC on PMI in CABG patients receiving cardioplegia or ICCF (274) and those on CABG with or without valve surgery receiving cardioplegia only (291), when our Institute observed a significant PMI reduction of 43% and 42.4% respectively in preconditioned subjects. However, it is relevant to notice that in this second study, patients with DM were excluded in contrast with the first study and our current subgroup analysis, in which we included both diabetic and non-diabetic subjects corresponding to 31% and 69% respectively of the total cohort: whether the exclusion of patients with known DM would lead to different outcomes will be discussed later.

Importantly, in the context of CABG surgery alone, our preconditioning stimulus led to a significant PMI reduction when both cardioplegia and ICCF patients were included but not when ICCF subjects were excluded: in this regard, it also relevant to mention that, with such exclusion our cohort size was reduced from 111 to 81 cases

and therefore it is possible that the smaller cohort could have had a relevant impact on the final outcome.

Similarly, in another study including patients undergoing CABG only with cold blood cardioplegia (286), Rahman and colleagues failed to show any beneficial effect of RIPC on PMI: however, differently from our study, importantly they also included patients undergoing urgent CABG surgery and excluded diabetic patients. Crucially, while a total of 162 patients were recruited into this study, our retrospective analysis only included 81 patients, of whom 25 were diabetic: our study was not powered for this type of analysis and therefore the ERICCA trial will ultimately give us the answer as to whether an enhanced preconditioning stimulus can protect high risk patients undergoing CABG with or without valve surgery with blood cardioplegia and improve their clinical outcomes. In the other study involving CABG patients only with cold blood cardioplegia and ICCF, (294), a strict anaesthetic regime had been used, however with a failure to show beneficial effects of RIPC on PMI. Interestingly, volatile agents had been used in the study by Rahman et al (286), which similarly showed no RIPC mediated cardioprotection but not in the studies from our group (282, 291) and Thielmann (293, 298, 300). Previous RCTs demonstrated that inhalant anaesthetics reduce PMI and mortality in patients undergoing cardiac surgery (460, 471, 472): therefore Karuppasamy and colleagues concluded that the reason for their negative findings could be attributed to the use of inhalant agents which may have optimised cardioprotection and therefore the addition of the benefits provided by RIPC was essentially not significant (294). Similarly, Lucchinetti et al (299) failed to show RIPC-induced cardioprotection in CABG patients receiving cardioplegia and isoflurane for anaesthesia maintenance and Kottenberg and colleagues (298) observed improved myocardial preservation only when RIPC was applied with the administration of

isoflurane and not propofol, although these patients received crystalloid and not blood cardioplegia.

Importantly, in contrast with the hypotheses generated from these studies, we have demonstrated that in the context of a combined use of volatile agents and propofol, RIPC improves myocardial preservation and decreases post-operative AF incidence: in the tertiary centre where we conducted our study, it is common practice to induce anaesthesia with a combination midazolam, fentanyl, anti-nicotinic agents and propofol/etomidate, whereas maintenance is guaranteed by the use of volatile anaesthetics and propofol. Indeed, in the subgroup analysis including CABG patients only, propofol was used in 90.2% of patients in the induction phase and 100% of cases in the maintenance phase, whereas either isoflurane or sevoflurane were administered exclusively during maintenance alongside propofol in all patients. Similarly in the subgroup including CABG and cardioplegia patients only, propofol was given to 91.9% of patients during induction and 100% during maintenance, whereas volatile agents were given only during maintenance and to all the patients included. It is therefore unlikely that the use of inhalant agents might have had a significant impact on the outcomes of the studies considered, which crucially used a standard upper limb preconditioning stimulus in contrast with our study using multi-limb IR, therefore confirming our hypothesis that an enhanced RIPC stimulus reduces PMI in patients undergoing CABG surgery with or without valve surgery.

Additionally, of the published studies using cold crystalloid and not blood cardioplegia (293, 295, 297, 298, 300), Wagner and colleagues only showed reduced mean TnI at 8 hours post-CPB with a preconditioning stimulus applied the day prior to surgery (295) and Lomivorotov et al (297) showed no cardioprotection provided by RIPC in a small population of low risk patients whose mean EuroSCORE was 2.2±0.6

and 2.5±0.8 respectively in control and preconditioned groups, and for whom therefore the additional benefit provided by RIPC potentially might have not been significant. Conversely, Thielmann and colleagues, on two separate RCTs (293, 300), demonstrated first in a small group of 53 non-diabetic patients (293) and then in a large cohort of 329 diabetic and non-diabetic patients that RIPC could reduce PMI (respectively of 45% and 17.3%). Interestingly, whilst our hsTnT reduction of 23% in CABG was statistically significant, the one of 18% in CABG patients receiving cardioplegia only did not reach statistical significance despite being superior to that observed in the most recent study from Thielmann and colleagues (300). Moreover, in the latter work, the authors could also importantly demonstrate an improvement in clinical outcomes at 1 year, with a significant reduction of all-cause mortality and MACCE rate, mainly driven by reduced MI events and with no significant difference in the incidence of stroke, repeat revascularisation, and cardiac death. Of note, our smaller RCT showed no difference in MACCE at 6 weeks follow-up. Another important difference between our study and the most recent RCT from Thielmann and co-workers is again the type of myocardial preservation used: in our proof-of-concept trial, we used either blood cardioplegia or ICCF, differently from the study from Thielmann et al, which used cold crystalloid cardioplegia only: the implications of blood versus crystalloid cardioplegia on PMI in cardiac surgery have already been extensively described in the previous section.

In summary, as previously discussed the inconsistent findings deriving from the outcomes of the different proof-of-concept RCTs are very likely to be related to a number of potential factors, including intervention protocols, confounding comorbidities, concomitant pharmacological therapy, anaesthetic regimens, surgical techniques and intra-operative methods of myocardial protection, and each of these

factors can individually have an impact on the final magnitude of RIPC-induced cardioprotection.

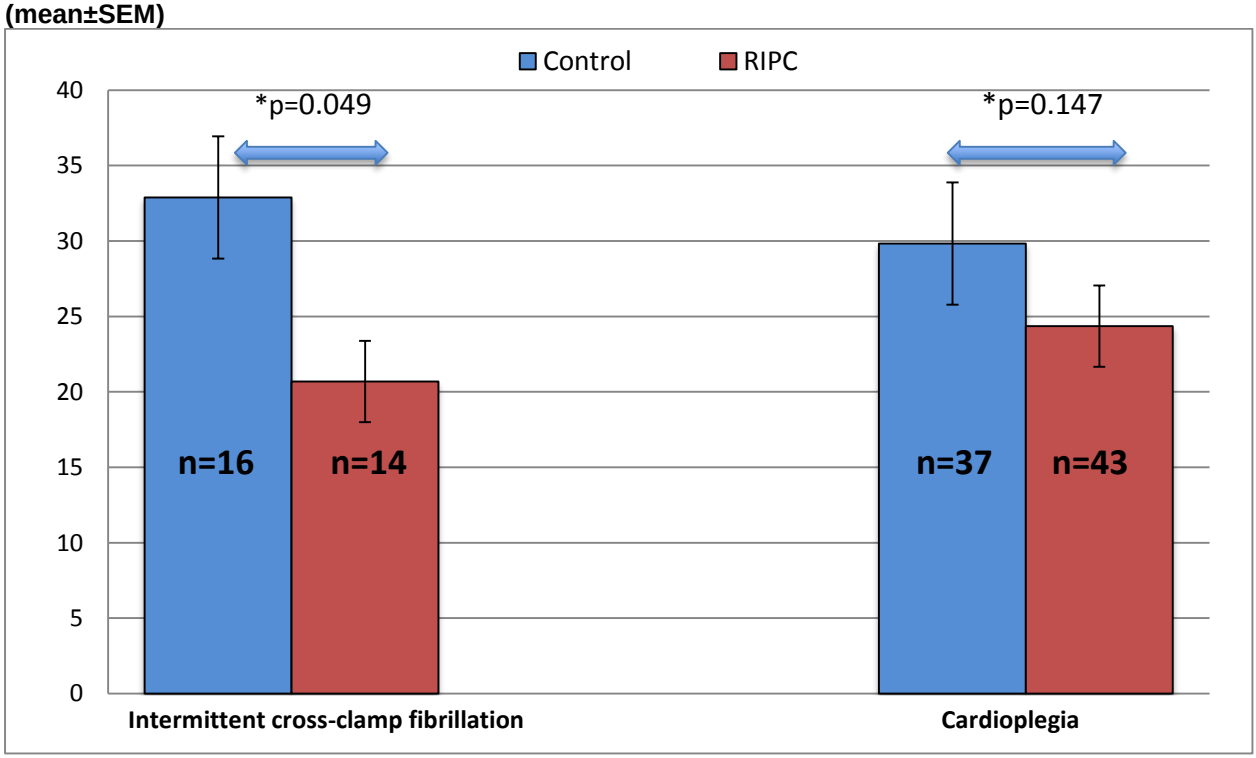
Importantly, we have found that in control patients total 72 hours hsTnT AUC was not significantly different between those receiving cardioplegia and those having ICCF (respectively 37.089±25.730 µg/L and 32.885±18.771 µg/L [4.204; CI -9.369-17.778; p=0.540], although CPB and cross-clamp times were significantly lower in the ICCF group (100.44±33.12 min versus 77.21±21.98 min [23.22; CI 8.75-37.69; p=0.003] and 70.56±24.44 min versus 33.00±7.49 min [37.56; CI 30.46-44.65; p<0.001] respectively. However, whilst the cardioplegia group included patients receiving different types of cardiac surgery including CABG with or without AVR, AVR only, MV surgery and AVR plus MVR with understandably prolonged CPB and cross-clamp times, ICCF was only used in patients undergoing CABG surgery alone. We therefore intended to perform a retrospective analysis to assess PMI magnitude in control patients undergoing CABG only and receiving either cardioplegia or ICCF to then evaluate the potential benefit provided by the application of our preconditioning stimulus **(Table 3.28)**. In the first instance we found that in control ICCF patients, hsTnT AUC was only marginally increased to 10% compared to control cardioplegia subjects 29.832±19.206 µg/L to 32.885±18.771 µg/L [-3.053; CI -14.618, 8.408; p=0.593], therefore confirming similar findings from previous studies (31, 89-93) showing that the overall PMI magnitude is not different between subjects receiving the two techniques of myocardial preservation **(Table 3.28, Fig. 3.14)**. As previously discussed, this is due to the fact that the potential more severe ischaemic damage induced by ICCF might be compensated by its significantly shorter ischaemic times compared to cardioplegia, which conversely provides significant myocardial cell protection at the expense of more prolonged ischaemic times.

Table 3.27. AUC, EuroSCORE, CPB and cross-clamp times in patients undergoing CABG surgery only and receiving cardioplegia or ICCF

Parameters		Cardioplegia Control: n=37 RIPC: n=43 (mean (SD))	ICCF Control: n=16 RIPC: n=14 (mean (SD))	Difference (95% CI)	P value
AUC	Control	29.832 (19.206)	32.885 (18.771)	-3.053 (-14.618, 8.408)	0.593
	RIPC	24.355 (13.052)	20.692 (6.039)	3.663 (-4.490, 11.816)	0.371
EuroSCORE	Control	3.37 (1.87)	2.69 (1.70)	0.68 (-0.41, 1.77)	0.215
	RIPC	3.14 (2.34)	1.93 (2.2)	1.21 (-0.24, 2.66)	0.100
CPB time	Control	90.29 (30.79)	77.21 (21.98)	13.08 (-4.98, 31.13)	0.152
	RIPC	87.30 (23.20)	77.30 (15.1)	10.02 (-3.28, 23.31)	0.137
Cross-clamp time	Control	62.61 (21.86)	33.00 (7.49)	29.61 (17.11, 42.10)	<0.001
	RIPC	61.21 (17.66)	35.30 (7.10)	25.92 (16.18, 35.67)	<0.001

RIPC=remote ischaemic preconditioning; SD=standard deviation; ICCF=intermittent cross-clamp fibrillation, CI=confidence interval; AUC=area under the curve; CPB=cardio-pulmonary bypass

Fig. 3.14. Comparison of AUC in control and RIPC patients receiving either ICCF or cardioplegia (mean±SEM)



RIPC=remote ischaemic preconditioning; ICCF=intermittent cross-clamp fibrillation; AUC=area-under-the-curve; SEM=standard error of the mean. *Unpaired Student T-Test

It is has been demonstrated that ischaemic times are correlated to PMI in cardiac surgery (473, 474): these refer to the period of time during which the myocardium is deprived of blood supply and in the context of on-pump cardiac surgery and correspond to aortic cross-clamp times, during which coronary perfusion is interrupted. Ischaemic times are independent predictor of PMI in cardiac surgery and aortic cross-clamp times longer than 100 min have been associated with significant PMI in CABG patients (474). In a more recent study, aortic cross-clamp time greater than 90 minutes and CPB time greater than 180 minutes, were also independent predictors of myocardial damage (473). In our subgroup analysis we have demonstrated that PMI magnitude was not significantly changed in control patients receiving ICCF and therefore subjected to significantly shorter ischaemic times than those having cardioplegia. Crucially the application of our enhanced preconditioning stimulus to CABG patients only led to significant PMI reduction in patients receiving ICCF: whilst this could be well related to the relatively small cohort size, it is also valuable to note that in preconditioned patients, the use of ICCF was not associated with a significantly lower PMI: whether the beneficial effects provided by ICCF in preconditioned patients may be less significant as cardioprotection may have already been “optimised” by our RIPC stimulus is difficult to know and certainly larger studies would be able to confirm this.

3.13.2.5. Effects of multi-limb RIPC on cardioprotection in patients undergoing valve surgery

As with studies on CABG with or without valve surgery, previous RCTs on valve surgery alone have showed conflicting results and therefore it is still not unclear whether RIPC provides a beneficial cardioprotective effect on these patients. The combination of high mean EuroSCORE, CPB times and cross-clamp times categorises these subjects into a high peri-operative risk category: we therefore conducted a further retrospective analysis in order to establish whether an enhanced preconditioning stimulus reduces PMI and improves clinical outcomes in these subjects.

A total of 48 patients underwent valve surgery alone, including either AVR or MV repair or replacement, of which 25 randomised to control and 23 to RIPC **(Table 3.29)**. We found no significant difference in patients' baseline characteristics or details of surgery, except for the use of intra-operative GTN, which was higher in the sham group, with 14 patients in the sham group versus 6 in the RIPC group receiving iv GTN, corresponding to respectively 56% and 26% of subjects. **(Tables 3.29-3.30)**. Importantly all these patients received cardioplegia for myocardial preservation.

Table 3.28. Patient baseline characteristics in patients undergoing valve surgery alone

Patients	Control (n=25) (mean (SD))	RIPC (n=23) (mean (SD))	P value
Age	65±13	66±12	0.836
Gender			0.566
Male	14 (56.0%)	15 (65.2%)	
Female	11 (44.0%)	8 (34.8%)	
Ethnicity			0.122
Caucasian	23 (92.0%)	18 (78.3%)	
Asian	1 (4.0%)	0 (0.0%)	
Afro-Caribbean	1 (4.0%)	5 (21.7%)	
Chinese	0 (0.0%)	0 (0%)	
BMI	27.2±6.3	28.2±5.6	0.980
SBP *(mmHg)*	130.2±17	130.3±12.1	0.555
DBP *(mmHg)*	72.0±10.0	73.1±11.2	0.329
HR *(bpm)*	67.1±11.5	68.9±9.4	0.124
Smoking History			0.334
Smoker	2 (8.0%)	2 (8.7%)	
Ex-smoker	31 (57.4%)	31 (54.4%)	
Non-smoker	16 (64.0%)	10 (43.5%)	
Family History of IHD	12 (48.0%)	13 (56.5%)	0.578
NYHA Class	3.20±0.9	2.82±0.73	0.124
CCS Class	1.28±0.74	1.45±1.06	0.511
LVEF			0.378
>50%	22 (88.0%)	21 (71.9%)	
30%-50%	3 (12.0%)	1 (4.3%)	
<30%	0 (0.0%)	1 (4.3%)	
Co-morbidities			
Diabetes Mellitus	3 (12.0%)	4 (17.4%)	0.696
Hypertension	16 (64.0%)	15 (65.2%)	1.000
Hypercholesterolemia	8 (32.0%)	14 (60.9%)	0.081
Atrial Fibrillation	10 (40.0%)	8 (34.8%)	0.772
Previous MI	1 (4.0%)	1 (4.3%)	1.000
Previous PCI	1 (4.0%)	1 (4.3%)	1.000
Previous CVA/TIA	3 (12.0%)	1 (4.3%)	0.383
Previous Cardiac Surgery	2 (8.0%)	3 (13.0%)	0.794
Other comorbidities	3 (12.0%)	1 (4.3%)	0.502
Peripheral Arterial Disease	0 (0.0%)	0 (0.0%)	1.000
Drug History	14 (56.0%)	11 (50.0%)	0.773
Aspirin	4 (16.0%)	0 (0.0%)	0.112
Clopidogrel/Prasugrel	1 (4.0%)	1 (4.3%)	0.845
Warfarin	9 (36.0%)	12 (54.5%)	0.256
Beta-blocker	7 (28.0%)	7 (31.8%)	0.626
Calcium Channel Blocker	14 (56.0%)	15 (63.6%)	0.098
Statin	13 (52.0%)	12 (54.5%)	0.168
ACE-I/ARB	1 (4.0%)	0 (0.0%)	1.000
Long acting nitrates			
Antidiabetics	0 (0.0%)	1 (4.3%)	0.851
Insulin	0 (0.0%)	2 (8.6%)	0.222
Biguanide	1 (4.0%)	1 (4.3%)	1.000
Sulphonylurea	11 (44.0%)	11 (50.0%)	0.666
Diuretics			

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA=Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF=left ventricular ejection fraction.

Table 3.29. Details of surgical procedure in patients undergoing valve surgery alone

Patients	Control (n=25) (mean (SD))	RIPC (n=23) (mean (SD))	P value
Indication for Surgery			0.741
Valve Disease	23 (92.0%)	22 (95.6%)	
SBE	2 (8.0%)	1 (4.4%)	
Angina and Valve Disease	1 (4.0%)	0 (0.0%)	
EuroSCORE	4.60±2.22	5.00±2.07	0.522
Additive perioperative risk			0.337
Low (EuroSCORE 0-2)	3 (12.0%)	4 (17.4%)	
Medium (EuroSCORE 3-5)	14 (56.0%)	8 (34.8%)	
High (EuroSCORE >5)	8 (32.0%)	11 (47.8%)	
Bypass-time (min)	103.76±29.95	88.32±31.99	0.094
Cross-clamp time (min)	73.88±22.49	65.68±26.34	0.261
Cardioprotection			
Blood cardioplegia	25 (100%)	23 (100%)	1.000
Anesthetic agents			
Induction			
Anti-nicotinic agents			0.570
Rocuronium	22 (91.7%)	20 (90.9%)	
Pancuronium	2 (4.2%)	1 (4.5%)	
Vecuronium	0 (0.0%)	1 (4.5%)	
Midazolam	14 (58.3%)	6 (27.3%)	0.420
Etomidate	1 (4.0%)	1 (4.5%)	1.000
Fentanyl	25 (100%)	23 (100%)	1.000
Propofol	24 (96.0%)	22 (95.5%)	1.000
Maintenance			
Propofol	25 (100%)	21 (95.5%)	1.000
Volatile Anesthetics			1.000
Isoflurane	24 (96.0%)	51 (94.4%)	
Sevoflurane	1 (4.0%)	1 (4.5%)	
Intra-operative GTN	14 (56.0%)	6 (26.1%)	0.045

RIPC=Remote Ischemic Preconditioning; CABG=Coronary artery bypass graft; AVR=Aortic valve replacement.

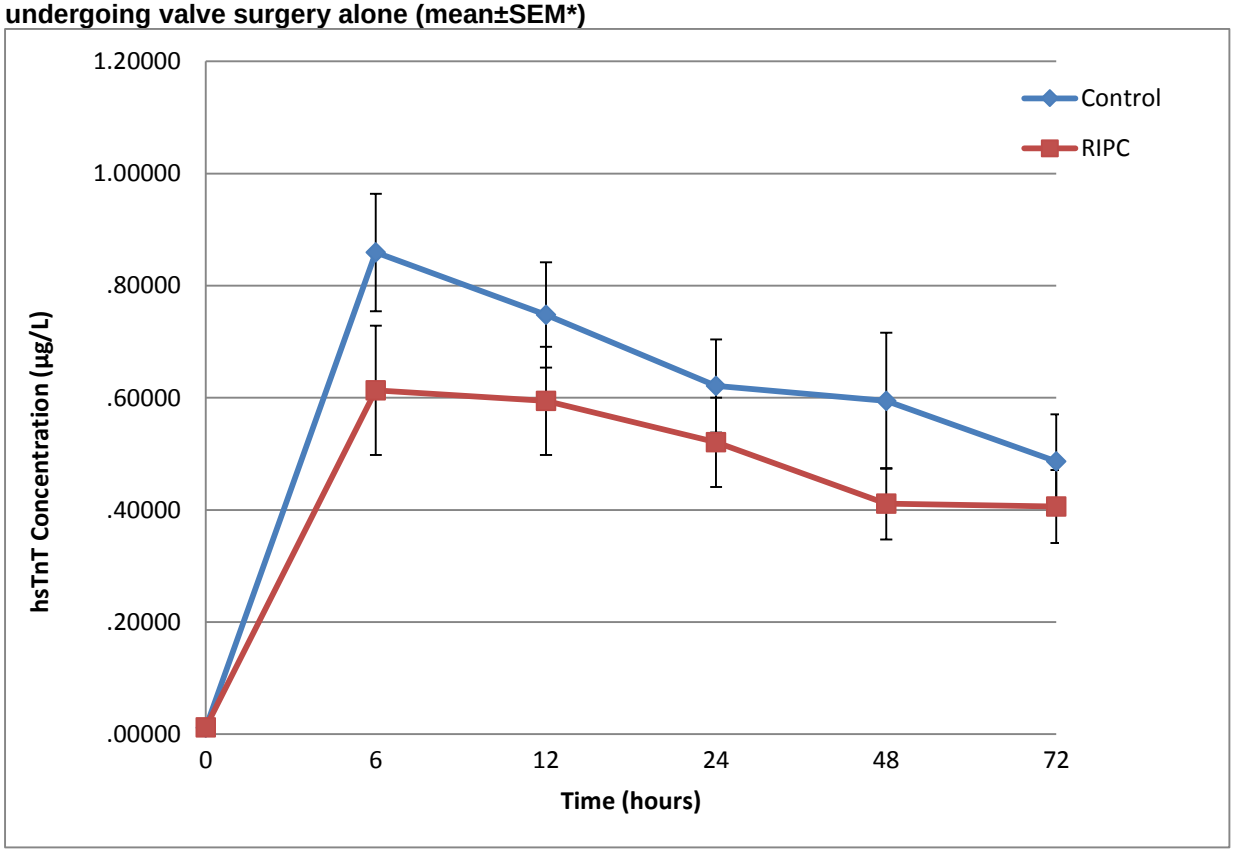
Despite a reduction of mean hsTnT levels in the preconditioned group at all the specified time-points, this did not reach statistical relevance **(Table 3.31, Fig. 2.15)**. Similarly, RIPC reduced total hsTnT AUC from 43.925±33.144 μg/L to 33.395±23.719 μg/L, corresponding to a non-significant 24% decrease of hsTnT release in the 72 post-operative hours [10.529; CI -6.868, 27.927; p=0.229]**(Table 3.31, Fig. 2.16)**.

Table 3.30. High-sensitivity Troponin-T release at the specified time point in patients undergoing valve surgery alone

Endpoint	Control (n=25) (mean (sd))	RIPC (n=23) (mean (sd))	Difference (95% CI)	P value
hsTnT *(µg/L)*				
Pre-operatively	0.012 (0.019)	0.012 (0.014)	-0.000 (-0.010, 0.009)	0.927
6 hours post-operatively	0.859 (0.523)	0.613 (0.552)	0.246 (0.066, 0.588)	0.120
12 hours post-operatively	0.748 (0.469)	0.594 (0.462)	0.153 (0.117, 0.424)	0.260
24 hours post-operatively	0.621 (0.607)	0.411 (0.298)	0.101 (-0.133, 0.334)	0.390
48 hours post-operatively	0.486 (0.404)	0.406 (0.305)	0.183 (-0.103, 0.471)	0.205
72 hours post-operatively	0.378 (0.325)	0.232 (0.161)	0.080 (-0.135, 0.296)	0.457
Total 72 hours AUC	43.925 (33.144)	33.395 (23.719)	10.529 (-6.868, 27.927)	0.229

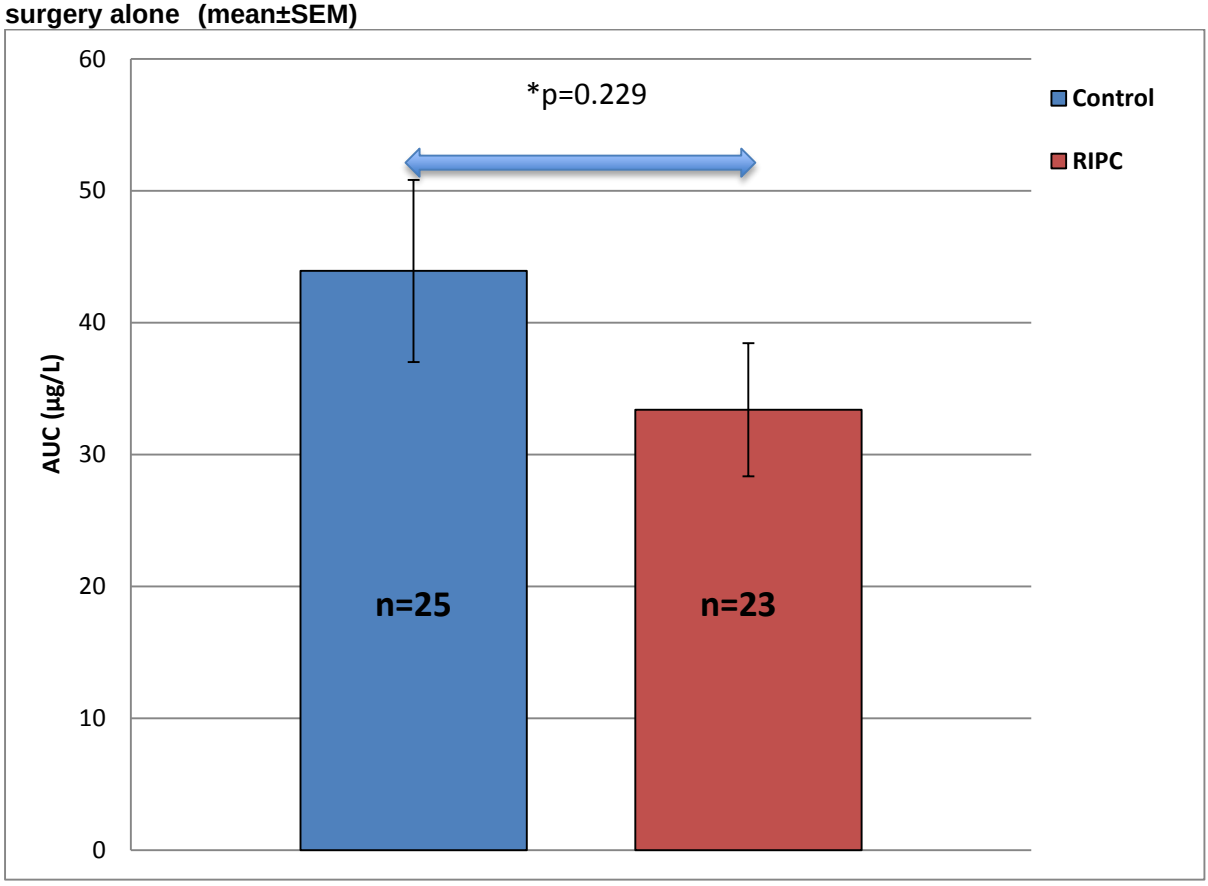
RIPC=Remote ischemic preconditioning; SD=standard deviation; CI=confidence interval; hsTnT=high sensitivity Troponin-T

Fig. 3.15. High-sensitivity Troponin-T at 0, 6, 12, 24, 48 and 72 hours post-operatively in patients undergoing valve surgery alone (mean±SEM*)



RIPC=remote ischaemic preconditioning; hsTnT=high-sensitivity Troponin-T; SEM=standard error of the mean. *Unpaired Student T-Test

Fig. 3.16. Total Area under the Curve of high-sensitivity Troponin T in patients undergoing valve surgery alone (mean±SEM)



RIPC=remote ischaemic preconditioning; AUC=area under the curve
* Unpaired Student T-Test

With regards to secondary end-points, we observed a reduction of 50% of total AKI cases and 35% of total inotrope requirement although again no statistical significance was reached **(Table 3.32)**. The remaining study outcomes were essentially comparable between the two intervention groups.

Table 3.31. Summary of major secondary endpoints in patients undergoing valve surgery alone

Endpoint	Control (n=54) (mean (sd))	RIPC (n=57) (mean (sd))	Difference (95% CI)	P value
CK (*μg/L*)				
Total AUC	28078.06 (10857.95)	34964.63 (29810.70)	-6886.57 (-22625.51, 8852.37)	0.395
Creatinine (mg/ml)				
Pre-operatively	76.72 (14.16)	84.22 (28.39)	-7.40 (-20.37, 5.38)	0.262
24 hours post-operatively	86.84 (27.43)	87.96 (28.52)	-1.12 (-17.38, 15.14)	0.891
48 hours post-operatively	89.56 (41.89)	94.39 (47.35)	-4.83 (-30.76, 21.10)	0.709
72 hours post-operatively	84.64 (38.44)	95.70 (57.38)	-11.06 (-39.22, 17.11)	0.434
Urine Output (ml)				
24 hours post-operatively	2056.3 (1040.9)	2105.86 (534.9)	-49.54 (-546.2, 447.1)	0.842
48 hours post-operatively	1996.3 (812.2)	2336.9 (911.5)	-340.6 (-885.3, 204.2)	0.214
72 hours post-operatively	2084.8 (701.4)	2485.5 (806.5)	-400.7 (-998.5, -197.2)	0.180
Total	5829.1 (1470.0)	6570.3 (1852.4)	-741.2 (-2079.4, 597.1)	0.259
AKI score				
0	19	20		0.246
1	3	2		
2	3	0		
3	0	0		
Acute Kidney Injury	6	3		0.466
Inotrope score				
Post bypass	9.62 (17.81)	8.13 (14.61)	1.48 (-8.17, 11.14)	0.758
24 hours post-operatively	14.44 (24.21)	10.00 (15.84)	4.43 (-7.78, 16.63)	0.469
48 hours post-operatively	9.44 (17.88)	6.34 (12.87)	3.09 (-6.29, 12.49)	0.510
72 hours post-operatively	5.66 (12.48)	3.97 (14.79)	1.69 (-6.32, 9.70)	0.673
Total	38.94 (61.39)	25.48 (40.96)	13.46 (-17.66, 44.58)	0.377
New onset AF	3	3		1.000
Length of ICU stay (days)	3.0 (2.0 – 5.0)**	3.0 (3.0– 4.0)**		0.706***
Length of hospital stay (days)	10.0 (8.0 – 13.0)**	11.0 (9.0– 17.0)**		0.534***
Clinical outcomes at six weeks				
Death	2	0		0.499
Myocardial infarction	0	0		1.000
Stroke	0	1		0.211
Revascularization	0	0		1.000

RIPC=Remote ischemic preconditioning; CK=Creatinine Kinase; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve

**Results shown as median (inter-quartile range); *** P-value for Mann-Whitney-Wilcoxon test

We obtained similar results when, within this patient groups, we went on to analyse the 29 subjects undergoing AVR only, of whom 15 were randomised to control and 14 to RIPC; furthermore, 17 patients, 9 control and 8 RIPC, received MV surgery, with 14 undergoing surgical repair and 3 undergoing replacement: we did not proceed to analyse data from MV surgery patients only as the sample size was too small for any relevant finding. No significant difference was found between control and RIPC groups within AVR surgery subjects with regards to baseline characteristics and surgical parameters **(Tables 2.33-2.34)**. In particular, CPB and cross-clamp times were longer in the control AVR group, however this did not reach statistical significance. Interestingly, intra-operative use of GTN was not statistically different between the intervention groups.

Mean hsTnT levels were lower in the preconditioned patients compared to control, however with no statistical relevance. Our enhanced preconditioning stimulus reduced the total hsTnT AUC from 38.499±37.661 μg/L to 27.947±24.678 μg/L [10.55; CI -14.96, 36.06; p=0.402], which corresponded to a non-statistical significant reduction of 27% **(Table 2.35)**. Mean hsTnT was lower in the preconditioned group at all the post-operative time-points, however this did not reach statistical significance. Interestingly, we found for the first and unique time in our study, that hsTnT at 48 hours was higher than the preceding mean at 24 hours: it is difficult to explain this finding, although it is highly likely to be related to the small number of patients in this subgroup analysis and therefore to chance. Preconditioned and control patients had comparable AKI and similarly all the remaining secondary end-points were similar amongst the intervention groups **(Table 2.35).**

Table 3.32. Baseline characteristics of patients undergoing AVR alone

Patients	Control (n=15) (mean (SD))	RIPC (n=14) (mean (SD))	P value
Age (*years*)	64±13	67±13	0.551
Gender			0.518
Male	9 (60.0%)	10 (71.4%)	
Female	6 (40.0%)	4 (28.6%)	
Ethnicity			0.129
Caucasian	15 (100.0%)	12 (60.7%)	
Asian	0 (0.0%)	2 (14.3%)	
Afro-Caribbean	0 (0.0%)	0 (0.0%)	
Chinese	0 (0.0%)	0 (0.0%)	
BMI	29.5±6.5	29.1±5.4	0.892
SBP *(mmHg)*	131.8±16.4	135.9±13.9	0.604
DBP *(mmHg)*	72.3±11.3	77.5±10.8	0.226
HR *(bpm)*	68.6±11.9	70.2±8.9	0.702
Smoking History			0.193
Smoker	1 (6.7%)	1 (7.1%)	
Ex-smoker	12 (80.0%)	7 (50.0%)	
Non-smoker	2 (13.3%)	6 (42.9%)	
Family History of IHD	8 (53.3%)	9 (64.3%)	0.550
NYHA Class	3.13±0.7	2.85±0.8	0.334
CCS Class	1.33±0.7	1.38±0.9	0.866
LVEF			0.367
>50%	14 (93.3%)	13 (93.9%)	
30%-50%	1 (6.7%)	0 (0.0%)	
<30%	0 (0.0%)	1 (7.1%)	
Co-morbidities			
Diabetes mellitus	2 (13.3%)	2 (14.3%)	0.941
Hypertension	10 (66.7%)	11 (78.6%)	0.474
Hypercholesterolemia	7 (46.7%)	9 (64.3%)	0.340
Atrial Fibrillation	2 (13.3%)	6 (42.9%)	0.075
Previous MI	1 (6.7%)	1 (7.1%)	0.960
Previous PCI	1 (6.7%)	1 (7.1%)	0.960
Previous CVA/TIA	1 (6.7%)	1 (7.1%)	0.960
Previous Cardiac Surgery	0 (0.0%)	1 (7.1%)	0.292
Other comorbidities	2 (13.3%)	0 (0.0%)	0.139
Peripheral Arterial Disease	0 (0.0%)	0 (0.0%)	1.000
Drug History			
Aspirin	12 (80.0%)	6 (46.2%)	0.062
Clopidogrel/Prasugrel	2 (13.3%)	0 (0.0%)	0.172
Warfarin	1 (6.7%)	2 (15.4%)	0.542
Beta-blocker	6 (40.0%)	10 (76.9%)	0.602
Calcium Channel Blocker	6 (40.0%)	4 (30.8%)	0.615
Statin	10 (66.6%)	10 (76.9%)	0.536
ACE-I/ARB	17 (74.9%)	21 (75%)	0.797
Long acting nitrates	1 (6.7%)	0 (0.0%)	0.343
Antidiabetics			
Insulin	1 (6.7%)	1 (7.1%)	0.364
Biguanide	1 (6.7%)	1 (7.1%)	0.364
Sulphonylurea	1 (6.7%)	1 (7.1%)	0.916
Diuretics	7 (46.7%)	5 (38.5%)	0.909

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA= Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF= eft ventricular ejection fraction.

Table 3.33. Details of surgical procedure of patients undergoing AVR alone

Patients	Control (n=15) (mean (SD))	RIPC (n=14) (mean (SD))	P value
Indication for Surgery			0.617
Dyspnoea	1 (6.7%)	1 (7.1%)	
Valve Disease	13 (86.7%)	13 (92.9%)	
Infective Endocarditis	1 (6.7%)	0 (0.0%)	
EuroSCORE	4.13±1.84	5.14±1.99	0.168
Additive perioperative risk			0.381
Low (EuroSCORE 0-2)	2 (13.3%)	2 (14.3%)	
Medium (EuroSCORE 3-5)	9 (60.0%)	5 (35.7%)	
High (EuroSCORE >5)	4 (26.7%)	7 (50.0%)	
Bypass-time (min)	99.13±23.63	81.86±26.38	0.074
Cross-clamp time (min)	74.36±21.79	65.0±25.5	0.097
Cardioprotection			
Blood cardioplegia	15 (100%)	14 (100%)	1.000
Cross-clamp fibrillation	0 (0.0%)	0 (0.0%)	
Anesthetic agents			
Induction			
Anti-nicotinic agents			0.343
Rocuronium	14 (93.3%)	15 (100.0%)	
Pancuronium	1 (6.7%)	0 (0.0%)	
Vecuronium	0 (0.0%)	0 (0.0%)	
Midazolam	10 (66.7%)	3 (23.1%)	0.021
Etomidate	1 (6.7%)	0 (0.0%)	0.343
Fentanyl	15 (100%)	14 (100%)	1.000
Propofol	14 (93.3%)	15 (100.0%)	0.343
Maintenance			
Propofol	15 (100%)	14 (100%)	1.000
Volatile Anesthetics			
Isoflurane	15 (100%)	14 (100%)	1.000
Sevoflurane	0 (0.0%)	0 (0.0%)	
Intra-operative GTN	7 (46.7%)	2 (14.3%)	0.060

RIPC= Remote Ischemic Preconditioning; CABG=Coronary artery bypass graft

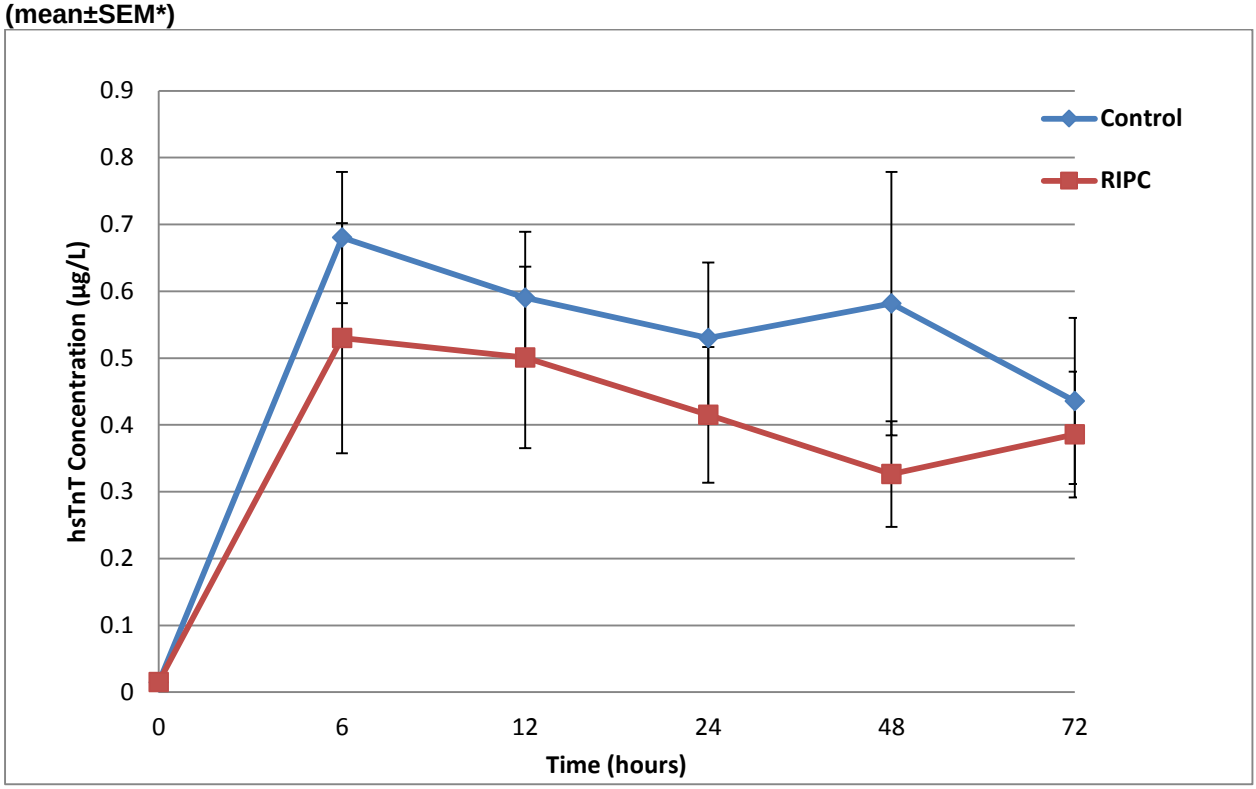
Table 3.34. Summary of major endpoints in patients undergoing AVR

Endpoint	Control (n=15) (mean (sd))	RIPC (n=14) (mean (sd))	Difference (95% CI)	P value
hsTnT *(μg/L)*				
Pre-operatively	0.0147 (0.024)	0.015 (0.017)	-0.0006 (0.08, -0.016)	0.937
6 hours post-operatively	0.680 (0.381)	0.529 (0.645)	0.151 (-0.252, 0.430)	0.447
12 hours post-operatively	0.590 (0.382)	0.501 (0.508)	0.089 (0.101, -0.053)	0.595
24 hours post-operatively	0.530 (0.438)	0.415 (0.366)	0.115 (-0.202, 0.431)	0.462
48 hours post-operatively	0.582 (0.763)	0.326 (0.285)	0.255 (-0.206, 0.717)	0.266
72 hours post-operatively	0.436 (0.465)	0.386 (0.339)	0.050 (-0.274, 0.375)	0.753
Total 72 hours AUC	38.499 (37.661)	27.947 (24.678)	10.55 (-14.96, 36.06)	0.402

CK (*µg/L*)				
Total AUC	26435.73 (10165.51)	35844.33 (35412.65)	-9408.60 (-32821.92, 14004.71)	0.410
Creatinine (mg/ml)				
Pre-operatively	77.80 (14.07)	84.50 (29.57)	--6.70 (-24.15, 10.75)	0.451
24 hours post-operatively	86.87 (24.69)	92.50 (33.39)	-5.63 (-27.91, 16.64)	0.608
48 hours post-operatively	91.00 (38.48)	103.86 (55.79)	-12.86 (-49.15, 23.44)	0.474
72 hours post-operatively	83.87 (29.39)	106.29 (68.24)	-22.42 (-61.97, 17.13)	0.255
Urine Output (ml)				
24 hours post-operatively	1998.1 (898.5)	2289.7.4 (553.9)	-291.56 (-882.87, 299.76)	0.320
48 hours post-operatively	2151.9 (933.3)	2576.7 (894.5)	-424.91 (-1203.0, 353.3)	0.270
72 hours post-operatively	2028.9 (906.2)	2566.2 (567.8)	-537.30 (-1481.5, 406.9)	0.236
Total	5912.6 (1719.3)	6903.5 (1812.2)	-990.90 (-3148.75, 1166.89)	0.337
AKI score				
0	12	12		0.341
1	1	2		
2	2	0		
3	0	1		
Acute Kidney Injury	3	3		1.000
Inotrope score				
Post bypass	9.86 (20.55)	4.65 (10.67)	5.21 (-7.83, 18.24)	0.419
24 hours post-operatively	7.96 (12.98)	5.66 (12.23)	2.31 (-7.54, 12.15)	0.634
48 hours post-operatively	4.17 (11.89)	4.73 (12.52)	-0.56 (-10.1, 8.9)	0.904
72 hours post-operatively	2.07 (4.06)	1.07 (3.46)	0.99 (-1.96, 3.95)	0.494
Total	23.45 (45.86)	15.85 (33.93)	7.60 (-24.17, 39.37)	0.627
New onset AF	3	1		0.598
Length of ICU stay (days)	2.0 (2.0–3.0)**	2.0 (2.0–3.0)**		0.747***
Length of hospital stay (days)	8.0 (7.0 –13.5)**	8.5 (8.0-10.0)**		0.780***
Clinical outcomes at six weeks				
Death	2	0		0.487
Myocardial infarction	0	0		1.000
Stroke	0	0		1.000
Revascularization	0	0		1.000

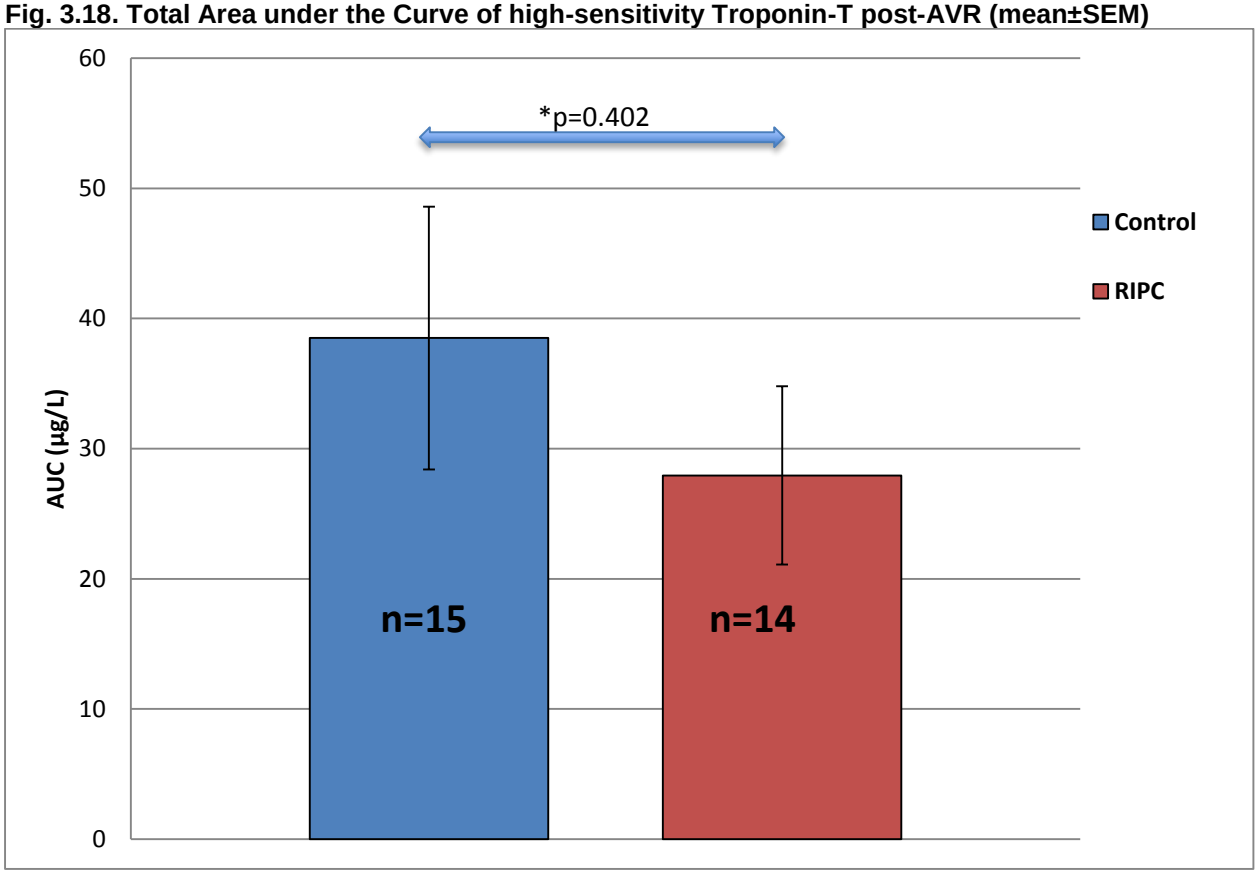
RIPC=Remote ischemic preconditioning; CK=Creatinine Kinase; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve; **Results shown as median (inter-quartile range). *** P-value for Mann-Whitney-Wilcoxon test

Fig. 3.17. Mean high-sensitivity Troponin-T levels at 0, 6, 12, 24, 48 and 72 hours post-AVR (mean±SEM*)



RIPC=remote ischaemic preconditioning; SEM=standard error of the mean. * Unpaired Student T-Test

Fig. 3.18. Total Area under the Curve of high-sensitivity Troponin-T post-AVR (mean±SEM)



RIPC=remote ischaemic preconditioning; AUC=area under the curve; SEM=standard error of the mean.
* Unpaired Student T-Test

3.13.2.5.1. Discussion

Our retrospective analysis showed that an enhanced preconditioning stimulus failed to demonstrate any protective effect on either PMI or short-term clinical outcomes in patients undergoing AVR.

In the first RCT investigating cardioprotection in the context of valve surgery alone (301), cardiac preconditioning reduced polymorphonuclear leukocytes, thromboxane B2, malonedialadehyde and pulmonary artery pressure and resistance and increased SOD and CGRP levels, pO2 and cardiac index. However the stimulus consisted of two-3 minutes cycles of aortic-cross clamping and 2 minutes of reperfusion before cardioplegic arrest, thereby representing an invasive strategy, in contrast with our protocol. Subsequently, three-minutes cycles of upper leg IR with a tourniquet after aortic cross-clamping were showed to reduce cTnI 5 minutes before declamping and 30 minutes after declamping, compared to the RIPC group, where the same stimulus was given after induction of anaesthesia, and the control group, where a sham tourniquet was applied around the upper thigh (302). However, no significant reduction of total cTnI AUC or clinical outcomes, including ventilation times, inotrope score, ICU and hospital stay, was achieved and despite a decrease in post-operative defibrillation rate. Based on these findings and combining the application of both RIPC and RIPostC, Kim and colleagues (305) evaluated the effects of three-10 minutes cycles of right lower limb ischaemia (at 250mmHg) 10 minutes after anaesthetic induction and from discontinuation of CPB in patients undergoing complex valve surgery: combined RIPC and RIPostC did not provide any significant benefit in pulmonary function and post-operative levels of IL-6, IL-8, IL-10 and TNF-α. However, in their study, they included a total of 27 patients undergoing MV replacement (MVR) plus TV annuloplasty, 13 patients receiving AVR plus ascending aorta replacement and 1

patient having CABG plus MVR, which we excluded from the current analysis. Moreover, no RIPC group alone or RIPostC group alone were included and no parameter related to cardioprotection was assessed (305). In the study from Young and co-workers (296), where complex cardiac operations were performed including high-risk CABG and combined CABG-valve surgery with an overall EuroSCORE of 7.1±6.1 and 6.6±6.1 in preconditioned and control patients respectively, subjects randomised to standard RIPC had higher hsTnT release and inotropic requirements. However, it is difficult to compare this study with our retrospective analysis as the former also included CABG patients and importantly only measured hsTnT levels at 6 and 12 hours, therefore failing to evaluate total hsTnT release over the 72 post-operative hours with AUC, which gives a true measure of total PMI magnitude.

In another study where RIPC was applied with three-10 minutes cycles of lower limb IR to patients undergoing complex valve surgery (303), Choi and colleagues found no difference in serum biomarkers levels of renal injury, although CK-MB was significantly decreased at 24 hours after surgery and ICU stay was reduced from 3.4±1.4 days to 2.7±0.7 days in the preconditioned group.

The most comparable RCT to our subgroup analysis included a total of 73 patients undergoing MV, AV or TV surgery (304), where anaesthesia was induced and maintained with sufentanil, etomidate and only in a small amount of patients with sevoflurane (no patient received propofol): standard RIPC reduced mean cTnI at 6, 12, 24, 48, and 72 hours and total AUC by 44% and improved NYHA and LVEF at 39 months follow-up. In our study, we found no statistically significant difference even when we combined the data from all valve operations. Importantly our study significantly differed from the work from Xie and colleagues (304) for the different type of anaesthetic regime used and more importantly for the smaller sample size (25

versus 35 patients in the control groups and 23 versus 38 patients in the RIPC groups): it is therefore possible that once again, anaesthetic regime might have had a significant impact on the cardioprotective effects of RIPC and that studies with standardised protocols will need to be carried out. Secondly, our study was not powered enough in order to evaluate the effects of RIPC in such a small population of patients undergoing valve surgery alone.

Additionally, patients receiving valve surgery only were not included in our multi-centre study and this was in order to evaluate a homogenous population were surgical parameters could be comparable between intervention groups (290). This implies that large RCTs are required in order to further evaluate the relationship between the intensity of the preconditioning stimulus and its effects on PMI in patients undergoing valve surgery alone.

CHAPTER 4

4. Effects of combined antegrade and retrograde cardioplegia on cardioprotection in patients undergoing cardiac surgery

4.1. Introduction

In chapter 1 we described the use of different techniques of myocardial preservation during cardiac surgery, comprising cardioplegia and ICCF, and throughout our analyses in chapter 3 we assessed the effects of our enhanced preconditioning stimulus in the setting of different types of operation and with specific methods of myocardial preservation: crucially, with the technique of cardioplegia, different types of solution, temperature and delivery have been developed throughout the decades, with specific centres and surgeons preferring one method versus the other.

The two most commonly used cardioplegic solutions are crystalloid and blood cardioplegia; with regards to the route of administration and temperature (21-24) the different combinations include: cold blood antegrade cardioplegia with topical cooling, warm blood antegrade cardioplegia, warm blood antegrade and retrograde combined cardioplegia, cold blood antegrade and retrograde combined cardioplegia, alternating cold or warm blood antegrade, retrograde combined cardioplegia and simultaneous cold or warm blood antegrade and retrograde combined cardioplegia. Despite significant distinctions, these methods are equally able to preserve myocardial function and therefore to limit PMI during cardiac surgery.

One of the most important aspects of the use of cardioplegia to achieve successful myocardial protection is the prompt delivery of the solution to all myocardial territories (475): in this regard, the most commonly used technique is the antegrade route, where cardioplegia is administered either into the aortic root or directly into the coronary ostia (476). Crucially, adequate delivery of the solution to cardiomyocytes occurs in the context of unobstructed coronary arteries and therefore the presence of significant coronary stenoses might compromise the homogenous distribution of the solution (477). This might render the post-stenotic myocardial territories particularly vulnerable to ischemia during aortic cross-clamp (478) and therefore resulting in substantial PMI and potential post-operative LV dysfunction (479).

Moreover, in the presence of significant aortic stenosis, the marked LV hypertrophy typical of these patients might once again limit the consistent delivery of the solution due the increased LV wall thickness (478) and when concomitant AR occurs, pressure at the aortic root level might prove insufficient to allow even perfusion of the cardioplegic solution through the myocardium thereby further increasing the potential risk of PMI (480, 481). Importantly, even increasing the infusion pressure at the aortic root has been demonstrated to be insufficient in this regard (482). Conversely, the use of the retrograde technique has offered the opportunity to overcome this drawback by the delivery of cardioplegic solution through the coronary sinus, the Thebesian veins and subsequently, and without hindrance, a trans-mural network of veins, which have no valves or, differently from the coronary arteries, any type of atherosclerotic lesions (483): this can therefore serve as a conduit for the delivery of cardioplegic solution in a more homogenous manner than what observed with obstructed or sub-obstructed coronary arteries (484, 485). Additionally, retrograde cardioplegia also offer the advantage of a better visualisation particularly in the context

of AVR as the catheter is placed distant from the AV (486). Nevertheless, crucial limitations of retrograde cardioplegia are identified in:

1. technical difficulties, related to the cannulation of the coronary sinus in itself, the balloon inflation which can often cause venous rupture, and the perfusion pressure (487, 488);
2. the potential inadequate supply of the RV and posterior septum by the myocardial venous system as the anterior cardiac veins supplying the RV are not directly connected to the coronary sinus (489, 490).

The combination of both methods of antegrade and retrograde cardioplegia is thought to overcome limitations inherent to both techniques and has now become an increasingly used method of myocardial preservation during cardiac surgery (491): interestingly, although both retrograde coronary sinus perfusion and antegrade perfusion have been studied individually in experimental trials and used in patients undergoing CABG surgery, little information is available on the direct comparison of PMI magnitude between the two different methods of cardioprotection.

At the tertiary centre where this study was carried out, cardioplegia was used as either antegrade cold blood cardioplegia or antegrade/retrograde warm blood cardioplegia (**Table 4.1**): we therefore conducted a retrospective analysis of control patients undergoing first time CABG surgery recruited in our principal study in order to determine whether the addition of retrograde cardioplegia to antegrade cardioplegia leads to similar or improved myocardial preservation in these patients. We did not attempt to carry out a similar analysis in other subgroups given the small population size and in order to compare equivalent cohort of subjects.

Table 4.1. Distribution of different types of operations according to technique of myocardial preservation and intervention

Operation		Antegrade Cardioplegia (%)	Intermittent cross-clamp fibrillation (%)	Antegrade/retrograde Cardioplegia (%)
CABG	Control	28 (32%)	16 (18%)	10 (11%)
	RIPC	27 (30%)	14 (16%)	16 (18%)
AVR	Control	13 (15%)	0 (0%)	2 (2%)
	RIPC	13 (15%)	0 (0%)	1 (1%)
CABG + AVR	Control	10 (11%)	0 (0%)	0 (0%)
	RIPC	6 (7%)	0 (0%)	3 (3%)
MV Surgery	Control	9 (10%)	0 (0%)	0 (0%)
	RIPC	6 (7%)	0 (0%)	2 (2%)

CABG=coronary artery bypass graft; AVR=aortic valve replacement; MV=mitral valve; RIPC=remote ischaemic preconditioning

4.2. Methods

As this type of analysis involved direct comparison of three subgroups, statistical analysis was in part different from the one presented elsewhere in this work. Comparison between exposure groups was made by including the exposure variable as a categorical variable in a linear regression model for approximately normally distributed endpoint variables. For very skewed endpoint variables the median T-test was used. Where continuous endpoint variables were measured over time a repeated measures linear regression model was fitted to measure the association between exposure variable and endpoint. The assumptions of the linear regression models were performed by analysis of residuals. Categorical data were analysed using Fisher's exact test. No adjustment for multiplicity has been made. Data were analysed using Stata version 12.1.

4.3. Results

A total of 44 control patients undergoing elective CABG surgery were included in this analysis: 28 received antegrade cold blood cardioplegia (**group 1**), 16 ICCF (**group 2**) and 10 antegrade/retrograde warm blood cardioplegia (**group 3**). With regards to baseline characteristics, group 3 had a lower rate of positive family history of CAD and previous PCI, whereas group 2 had a higher incidence of CVA prior to CABG surgery (**Table 4.2**): we found no other significant difference between the three groups (**Table 4.2**). However, when we then analysed the details of surgical procedures, expectedly, we found that cross-clamp times were significantly lower in group 2 than groups 1 and 3, however all the remaining parameters of surgery were similar amongst the 3 groups (**Table 4.3**).

Table 4.2. Patient baseline characteristics in control patients undergoing elective CABG surgery with antegrade/retrograde cardioplegia, antegrade cardioplegia or ICCF

Patients	Group 1 (n=28) (mean (SD))	Group 2 (n=16) (mean (SD))	Group 3 (n=10) (mean (SD))
Age	67±8	62±10	69±9
Gender			
Male	23 (82%)	12 (75%)	8 (80%)
Female	5 (18%)	4 (25%)	2 (20%)
Ethnicity			
Caucasian	21 (75%)	13 (81%)	9 (90%)
Afro-Caribbean	1 (4%)	1 (6%)	1 (10%)
Asian	5 (18%)	2 (13%)	0 (0%)
Chinese	1 (4%)	0 (0%)	0 (0%)
BMI	27.6±4.9	30.4±5.0	27.8±4.5
SBP *(mmHg)*	131.3±20.8	128.3±16.9	128.9±21.2
DBP *(mmHg)*	70.3±7.4	73.4±10.6	69.1±11.6
HR *(bpm)*	66.8±11.1	68.6±10.5	65.7±13.9
Smoking History			
Smoker	4 (14%)	4 (25%)	1 (10%)
Non-smoker	6 (21%)	4 (25%)	4 (40%)
Ex-smoker	18 (64%)	8 (50%)	5 (50%)
Family History of IHD	22 (79%)	13 (81%)	4 (40%)
NYHA Class			
0	2 (7%)	3 (21%)	2 (22%)
I	8 (29%)	5 (34%)	4 (44%)
II	17 (61%)	5 (34%)	2 (22%)
III	1 (4%)	1 (7%)	1 (11%)
IV	0 (0%)	0 (0%)	0 (0%)
CCS Class			
0	5 (18%)	2 (14%)	3 (33%)
I	4 (14%)	5 (36%)	0 (0%)
II	16 (57%)	6 (43%)	4 (44%)
III	2 (7%)	1 (7%)	1 (11%)
IV	1 (4%)	0 (0%)	1 (11%)
LVEF			
>50%	19 (68%)	12 (75%)	7 (70%)
30%-50%	8 (29%)	3 (19%)	3 (30%)
<30%	1 (4%)	1 (6%)	0 (0%)
Co-morbidities			
Diabetes mellitus	11 (39%)	6 (38%)	0 (0%)
Hypertension	25 (89%)	13 (81%)	7 (70%)
Hypercholesterolemia	25 (89%)	15 (94%)	8 (80%)
Atrial Fibrillation	3 (11%)	0 (0.0%)	2 (20%)
Previous MI	13 (47%)	3 (19%)	6 (60%)
Previous PCI	8 (29%)	1 (6%)	0 (0%)
Previous CVA/TIA	3 (11%)	0 (0%)	3 (30%)
Previous Cardiac Surgery	0 (0%)	0 (0%)	0 (0%)
Peripheral Arterial Disease	3 (11%)	1 (6%)	2 (20%)
Drug History			
Aspirin	3 (11%)	2 (14%)	0 (0%)
Clopidogrel/Prasugrel	2 (7%)	0 (0%)	0 (0%)
Warfarin	1 (4%)	0 (0%)	0 (0%)
Beta-blocker	22 (79%)	11 (79%)	8 (80%)
Calcium Channel Blocker	12 (43%)	3 (21%)	4 (40%
Statin	26 (93%)	13 (93%)	10 (100%)
ACE-I/ARB	20 (71%)	8 (57%)	6 (60%)
Long acting nitrates	7 (25%)	2 (14%)	3 (30%)
Antidiabetics			
Insulin	4 (14%)	2 (14%)	0 (0%)
Biguanide	2 (7%)	1 (7%)	0 (0%)
Sulphonylurea	5 (18%)	2 (14%)	0 (0%)
Diuretics	7 (25%)	4 (29%)	2 (20%)

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA=Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF=left ventricular ejection fraction.

Table 4.3. Details of surgical procedure in control patients undergoing elective CABG surgery with antegrade/retrograde cardioplegia, antegrade cardioplegia or ICCF

Patients	Group 1 (n=28) (mean (SD))	Group 2 (n=16) (mean (SD))	Group 3 (n=10) (mean (SD))
Indication for Surgery			
Angina	23 (82%)	13 (81%)	7 (70%)
Myocardial Infarction	5 (18%)	3 (19%)	3 (30%)
EuroSCORE	3.2±1.9	2.7±1.7	3.8±1.8
Additive perioperative risk			
Low (EuroSCORE 0-2)	13 (46%)	8 (50%)	1 (10%)
Medium (EuroSCORE 3-5)	12 (43%)	7 (44%)	7 (70%)
High (EuroSCORE >5)	3 (11%)	1 (6%)	2 (20%)
Bypass-time (min)	93.9±34.6	77.2±22.0	80.3±12.8
Cross-clamp time (min)	62.2±24.4	33.0±7.5	63.7±13.4
Number of grafts			
One	1 (4%)	0 (0.0%)	0 (0.0%)
Two	9 (32%)	3 (19%)	1 (10.0%)
Three	10 (36%)	10 (63%)	8 (80.0%)
Four	8 (29%)	3 (19%)	1 (10.0%)
Anesthetic agents			
Induction			
Anti-nicotinic agents			
Rocuronium	24 (86%)	12 (92%)	6 (60%)
Pancuronium	3 (11%)	1 (7%)	2 (22%)
Vecuronium	1 (4%)	0 (0.0%)	1 (11%)
Midazolam	12 (43%)	7 (54%)	6 (67%)
Etomidate	1 (4%)	2 (14%)	2 (22%)
Fentanyl	28 (100%)	14 (100%)	9 (100%)
Propofol	27 (96%)	12 (86%)	7 (78%)
Maintenance			
Propofol	28 (100%)	14 (100%)	9 (100%)
Volatile Anesthetics			
Isoflurane	25 (89%)	13 (93%)	9 (100.%)
Sevoflurane	3 (11%)	1 (7%)	0 (0.0%)
Intra-operative GTN	24 (89%)	14 (86%)	8 (89%)

GTN= glyceryl trinitrate.

Baseline pre-operative hsTnT levels were<0.02 µg/L and not significantly different between the 3 groups (**Fig. 2, Table 2**). There was evidence that mean hsTnT at 6 [-0.56; 95% CI: -0.78, -0.34; p<0.001] and 12 hours [-0.43, CI: -0.65, -0.21; p<0.001] was lower in group 3 than group 1 (**Fig. 2, Table 2**). Total 72 hr hsTnT AUC was lower in group 3 compared to group 1 [-16.55; CI -30.08, -3.01; p=0.018] with a slightly weaker evidence of lower hsTnT AUC in group 3 compared to group 2 [-15.13; CI -29.87, -0.39; p=0.044] and no significant difference between group 2 to group 1

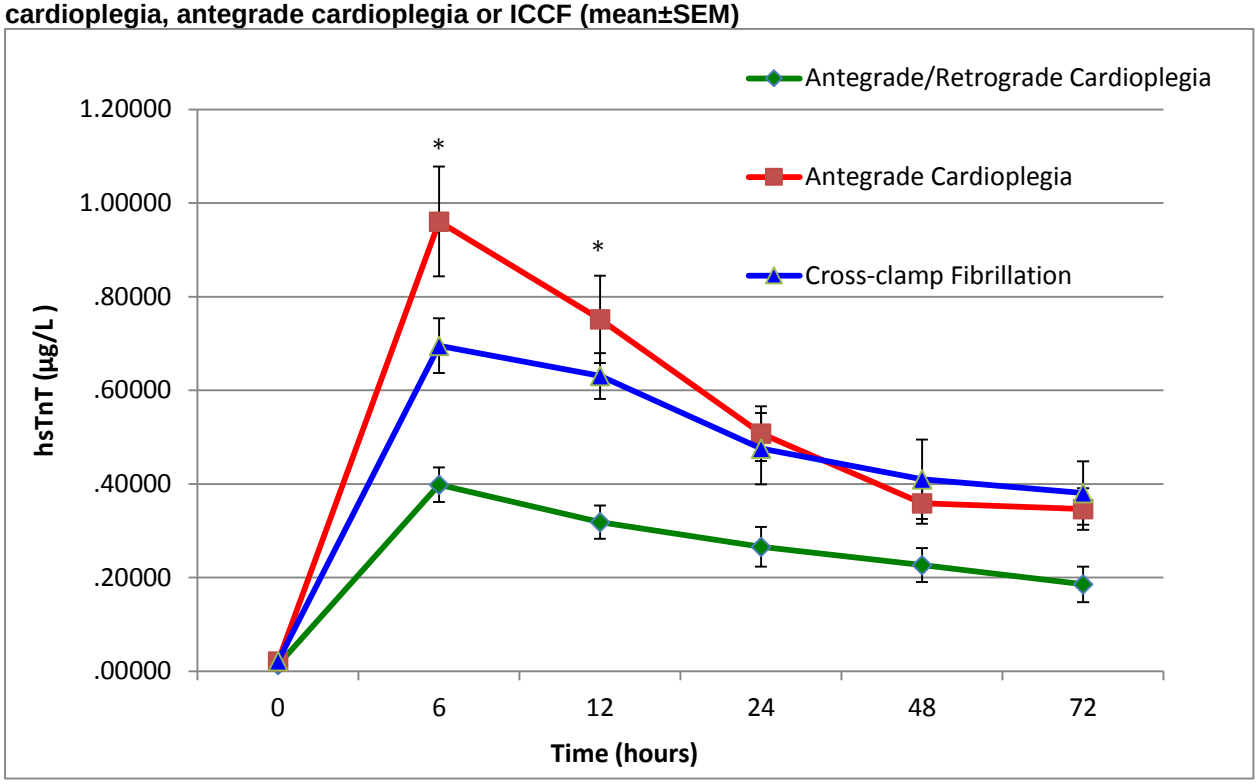
[-1.42; 95% CI: -12.95, 10.12, p=0.806] (**Fig. 4.2, Table 4.4**). No statistically significant difference was found amongst the three groups with regards to each of the secondary endpoints **(Table 4.3).**

Table 4.4. Mean high-sensitivity troponin-T at the specified time-points and total AUC in control patients undergoing elective CABG surgery with antegrade/retrograde cardioplegia, antegrade cardioplegia or ICCF

Patients	Group 1 (n=28) (mean [SD])	Group 2 (n=16) (mean [SD])	Group 3 (n=10) (mean [SD])		Comparison Group	Difference (95% CI)	Sub-group P value
Total 72 hours AUC	34.30 (20.35)	32.89 (18.77)	17.76 (7.54)	P= 0.050	1 vs 2 1 vs 3 2 vs 3	-1.42 (-12.95, 10.12) -16.55 (-30.08, -3.01) -15.13 (-29.87, -0.39)	0.806 0.018 0.044
Pre-operatively	0.021 (0.020)	0.022 (0.022)	0.014 (0.012)	P= 0.997	1 vs 2 1 vs 3 2 vs 3	0.000 (-0.188, 0.188) -0.008 (-0.229, 0.213) -0.008 (-0.250, 0.234)	
6 hours post-operatively	0.961 (0.619)	0.696 (0.235)	0.399 (0.117)	P<0.001	1 vs 2 1 vs 3 2 vs 3	-0.265 (-0.453, -0.077) -0.562 (-0.783, -0.341) -0.297 (-0.539, -0.055)	
12 hours post-operatively	0.752 (0.494)	0.631 (0.195)	0.319 (0.112)	P<0.001	1 vs 2 1 vs 3 2 vs 3	-0.121 (-0.309, 0.067) -0.433 (-0.654, -0.212) -0.312 (-0.557, -0.070)	
24 hours post-operatively	0.508 (0.309)	0.476 (0.305)	0.266 (0.134)	P= 0.100	1 vs 2 1 vs 3 2 vs 3	-0.032 (-0.220, 0.156) -0.241 (-0.463, -0.020) -0.209 (-0.451, 0.033)	
48 hours post-operatively	0.359 (0.224)	0.410 (0.339)	0.227 (0.114)	P= 0.335	1 vs 2 1 vs 3 2 vs 3	0.044 (-0.144, 0.233) -0.139 (-0.360, 0.083) -0.183 (-0.425, 0.059)	
72 hours post-operatively	0.347 (0.231)	0.381 (0.271)	0.186 (0.119)	P= 0.257	1 vs 2 1 vs 3 2 vs 3	0.027 (-0.161, 0.216) -0.168 (-0.390, 0.054) -0.195 (-0.437, 0.047)	

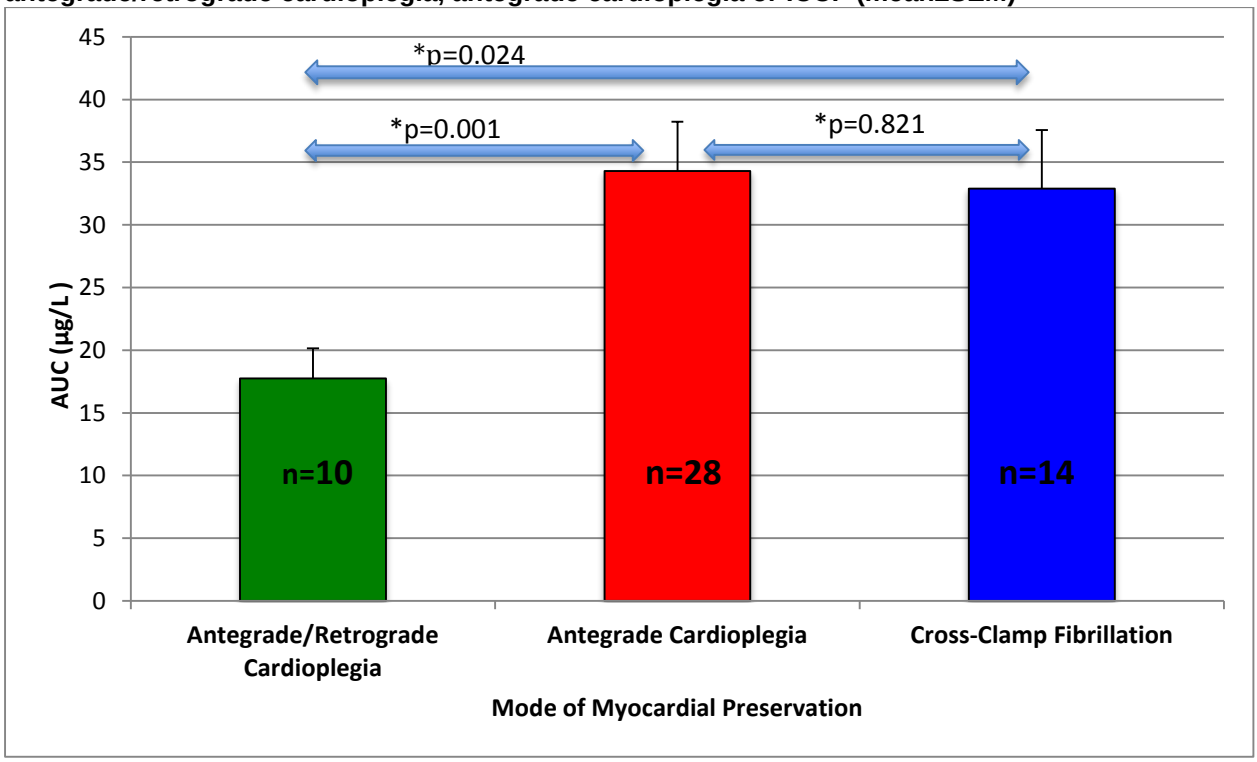
hsTnT=high sensitivity troponin-T; AUC=area-under-the-curve

Fig. 4.1: Mean high-sensitivity troponin-T pre-operatively and at 6, 12, 24, 48 and 72 hours post-surgery in control patients undergoing elective CABG surgery with antegrade/retrograde cardioplegia, antegrade cardioplegia or ICCF (mean±SEM)



hsTnT=high sensitivity troponin-T; ICCF=intermittent cross-clamp fibrillation; SEM=standard error of the mean. * p<0.05 (unpaired Student T-Test)

Fig. 4.2. Total hsTnT-AUC in control patients undergoing elective CABG surgery with antegrade/retrograde cardioplegia, antegrade cardioplegia or ICCF (mean±SEM)



AUC=area-under-the-curve; ICCF=intermittent cross-clamp fibrillation; SEM=standard error of the mean
*Unpaired Student T-Test

Table 4.5. Summary of secondary endpoints in control patients undergoing elective CABG surgery with antegrade/retrograde cardioplegia, antegrade cardioplegia or ICCF

Patients	Group 1 (n=28) (mean [sd])	Group 2 (n=16) (mean [sd])	Group 3 (n=10) (mean [sd])	P value
Creatinine (mg/ml)				
Pre-operatively	87.7±17.4	88.2±20.0	93.9±23.5	0.681
24 hours post-operatively	86.7±27.5	107.5±42.1	90.1±24.8	0.121
48 hours post-operatively	98.8±36.0	130.6±79.2	92.8±39.01	0.111
72 hours post-operatively	93.0±40.0	119.4±67.8	87.8±34.99	0.170
Urine Output (ml)				
24 hours post-operatively	1885.3±589.4	1941.9±806.7	2247.5±531.4	0.398
48 hours post-operatively	2274.1±1111.4	2033.1±951.3	2236.9±859.8	0.826
72 hours post-operatively	1912.0±852.7	2456.0±1138.1	2222.4±863.0	0.419
Total	5768.6±2187.1	6006.2±2080.0	6699.0±1485.4	0.686
Pre-operatively	87.7±17.4	88.2±20.0	93.9±23.5	0.681
AKI (N)				
0	22 (79%)	11 (69%)	10 (100.0%)	
1	5 (18%)	2 (13%)	(0.0%)	
2	0 (0.0%)	2 (13%)	(0.0%)	
3	1 (4%)	1 (6%)	(0.0%)	
Total number of AKI cases	6 (21%)	5 (31%)	0 (0.0%)	0.281*
Inotrope Score (mg/kg/hr)				
Post bypass	7.24 (13.79)	4.03 (11.92)	3.76 (4.35)	0.816**
24 hours post-operatively	11.90 (21.32)	10.11 (22.88)	3.67 (4.36)	0.635**
48 hours post-operatively	8.76 (15.39)	14.93 (33.57)	1.94 (2.98)	0.101**
72 hours post-operatively	1.85 (5.30)	13.54 (33.13)	2.13 (2.95)	0.015**
Total	29.88 (49.10)	42.54 (94.66)	11.66 (13.79)	0.545***
New onset AF (N)	6 (21%)	5 (31%)	4 (40.0%)	0.475*
Length of ICU stay (days)	2.0 (2.0-4.0)****	3 (1.0-7.5)****	2.0 (1.0-3.0)****	0.802*****
Length of Hospital stay (days)	8.5 (7.0-11.50)****	8.0 (6.0-10.50)****	7.5 (6.0-9.00)****	0.523*****
Clinical Outcomes at 6 weeks (N)				
Death	3 (14%)	0 (0.0%)	0 (0.0%)	0.283*
Myocardial infarction	1 (4%)	0 (0.0%)	0 (0.0%)	1.000*
Stroke	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000
Revascularization	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000*

sd=standard deviation; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit. *P value for Fisher's exact test; ** P-value from repeated measures linear regression model; *** P-value form linear regression model; **** Results shown as median (inter-quartile range); ***** P-value for Median T test.

4.4. Discussion

In our retrospective analysis we have found that the addition of retrograde cardioplegia to the antegrade technique reduces PMI compared to the use of antegrade cardioplegia alone or ICCF in control patients undergoing elective CABG surgery, although we found no significant impact on the study secondary outcomes.

Myocardial preservation during cardiac surgery is certainly one of the most debated topics in this field. A variety of myocardial protection strategies are currently used in the UK and the world and the choice of the type of technique, route and temperature of delivery is in the vast majority of cases at the surgeon's discretion as no consensus has yet been achieved on using a specific technique. However, the most commonly utilised technique by the majority of cardiac surgeons is the antegrade delivery of cardioplegia in which the solution is administered into the aortic root and spreads via the coronary arteries throughout the myocardium. Current clinical evidence favours the safety of this method, although the presence of severely stenosed coronaries in patients with advanced CAD can limit the uniform distribution of the cardioplegic solution, thereby exposing the myocardial areas not adequately reached by the solution to more severe ischemic injury during cardiac surgery (492). A proposed solution to this potential disadvantage is the retrograde route of delivery, with which cardioplegia is administered through the coronary sinus thereby relying on the extensive venous network of the heart and on the absence of atherosclerotic lesions compromising homogeneous cardioplegia distribution. In 1898 Pratts introduced the concept of the potential to "revive" an ischemic myocardium by supplying oxygenated blood through its venous system (493) and Blanco et al (486) in 1956, were the first group to successfully carry out a retrograde perfusion of the cardioplegia in the context of cardiac surgery: however, over the decades the concept

of retrograde technique has not become a widespread practice for myocardial protection in cardiac surgery for various reasons:

- the risk of vein rupture, which increases when the perfusion pressure into the coronary sinus is higher than the pressure in the venous system, which is between 30 and 40mmHg (494, 495);
- the catheter used for the solution delivery can cause mechanical injury of the coronary sinus or even its rupture and more worryingly the laceration of the atrioventricular groove due to balloon over-inflation or over-pressurised perfusion flow (496). This can be avoided by careful catheters inflation (496) and slow flow rates and pressures, which however are associated with significant ischaemic injury for the delay in arresting the heart when retrograde cardioplegia is used alone (497, 498);
- the presence of a large Thebesian valve, an embryological remnant of the sinoatrial valves located at the orifice of the coronary sinus, of which it covers more than 75% (499), can potentially interfere with the uniform administration of cardioplegia retrogradely (500);
- although still controversial, retrograde administration of cardioplegic solution may lead to inadequate delivery to RV and posterior septum, which can potentially expose the latter to more severe ischaemic injury (501).

The combined use of antegrade and retrograde techniques is potentially able to overcome the limitations presented by the two technique individually and to improve myocardial preservation in patients undergoing cardiac surgery: the outcomes of our retrospective study are strongly suggestive of this as they provide an objective evidence of a significant reduction of the total hsTnT release over the 72 post-

operative hours. This is therefore in contrast with the majority of previous studies which however have used the two techniques separately and not in combination: Menasche et al (481) first showed that cardiac outputs and RV-LV stroke indices were similar in patients receiving antegrade or cardioplegia during AVR. They then went on to retrospectively analyse clinical outcomes in a large cohort of patients undergoing either AVR alone or CABG plus AVR surgery and receiving retrograde cardioplegia only, thereby with no antegrade cardioplegia group for direct comparison (502): documented complications rate was 0.6% in a total of 500 patients, with an overall mortality incidence of 1.6%, which they found were similar to those of previously documented literature on antegrade cardioplegia only (502).

A further larger retrospective study was then conducted (503) on 1280 patients undergoing CABG surgery and/or valve repair/replacement and receiving antegrade cardioplegia followed by retrograde cardioplegia with shorter (less than 120 minutes) or longer aortic cross-clamping times (more than 120 minutes): crucially, despite a significantly higher number of combined CABG/AVR operations and reoperations in the long cross-clamp group, hospital mortality rates were similar between the two groups, although inotropic requirement, CK and CK-MB levels and hospital stay were lower in the short cross-clamp group. This importantly demonstrated that operations involving longer cross-times with antegrade followed by retrograde cardioplegia were equally safe than similar operations with shorter ischaemic times, in contrast with previous literature, which associated longer ischaemic times with worse patients' morbidity and mortality (504, 505).

Subsequently, in a small study on 20 patients undergoing CABG surgery randomised to either antegrade or retrograde cardioplegia (506), Kaukoranta et al showed that oxygen extraction, lactate production, adenosine catabolites analised

from myocardial biopsies of both ventricles, were higher in the RV of subjects of the retrograde group, which conversely, had higher total TnT and CK-MB release. Therefore they concluded that retrograde mild hypothermic blood cardioplegia leads to metabolic changes compatible with RV ischemia, albeit with preserved associated levels of high-energy phosphates, and uneventful postoperative course, and that this technique would need careful consideration particularly in patients with RV hypertrophy or dysfunction if used alone.

Crucially, in a study similar to ours involving 120 patients undergoing elective fist-time CABG surgery and comparing outcomes of subjects receiving antegrade cold blood cardioplegia with those having the combined technique (484), despite significantly longer infusion times in the antegrade/retrograde cohort, postoperative cardiac output, ECG changes, cardiac biomarkers, temporary pacing requirement and 30-day morbidity were similar in both groups. Similarly, in a RCT enrolling 87 patients undergoing CABG surgery (490), subjects receiving the combined technique had a 16.5% decrease of inotropic requirement, compared to those having antegrade cardioplegia only, although no difference was found in terms of patients' morbidity and mortality.

In our retrospective analysis we found no significant benefit of any of the study secondary endpoints amongst the three groups and similarly to the work from Radhemhr and colleagues, we intended to directly compare biochemical and clinical outcomes between patients receiving antegrade cardioplegia alone and the combined technique of antegrade/retrograde cardioplegia. In addition to this and for the first time in the literature, we also intended to compare these findings with those from patients receiving ICCF. In addition, we measured hsTnT concentrations at 6 different time points for all patients and calculated the total release with the 72-hours hsTnT AUC.

There is no study in our knowledge, that combines the following four factors including aortic cross-clamping times, combined versus antegrade cardioplegia alone versus ICCF, hsTnT levels at 6 different time-points with consequent total AUC and exclusively CABG patients. Furthermore, although increased cross-clamp times have been associated with worse PMI in cardiac surgery (484, 505), our study suggests that despite longer cross-clamp times, patients receiving combined antegrade/retrograde cardioplegia sustained less PMI compared to antegrade alone or ICCF alone, thereby indicating that the relationship between these two factors might be different and that cross-clamp time might be potentially less relevant than the type of myocardial protection used, as previously found by Bar-El et al (503). This is potentially crucial in complex cases where long cross clamp times are anticipated and/or patients are known to have poor LV function, for whom the best myocardial protection available would be warranted.

Another important aspect of our analysis was the different temperature employed between our two cardioplegia groups, with the antegrade method using cold blood and the combined technique using warm blood (see also chapter 1): the optimal temperature of cardioplegia during cardiac surgery is another crucial element of myocardial protection and it could be argued that the lower troponin rise in the combined group may be partially explained by the temperature difference. Cold cardioplegia is able to attenuate myocardial oxygen demand and the risk of ischaemic damage but conversely may lead to the inhibition of myocardial enzymes leading to a stunning of the metabolic and functional recovery following surgery. However warm blood cardioplegia is thought to counteract this potential deleterious effect. In a meta-analysis (507) involving 8814 patients randomised to either warm or cold cardioplegia predominantly in the setting of CABG surgery, no significant difference was found in

all-cause mortality or incidence of MI, IABP use, stroke, low-output syndromes and post-operative AF between the two groups and postoperative cardiac index was significantly improved in the warm blood cardioplegia group. Similarly, no difference was found in mortality, peri-operative MI, stroke or inotrope requirement between cold and tepid cardioplegia (508).

Our retrospective study has several limitations. The cohort population was small and additionally patients in group 3 were operated on by one consultant, with a subsequent potential bias. Typically in a study of this type strong prognostic and confounding variables would be adjusted for, however, the small sample size precluded detailed adjustment and we therefore acknowledge that some residual confounding bias may remain. Finally, we have not adjusted for multiplicity in our analysis and there is a possibility that the results may have arisen by chance. Moreover, our study suggests a significant PMI reduction with the addition of retrograde to antegrade cardioplegia, although we found no significant difference in any other outcome and therefore, whether the combined technique has an impact on patient morbidity and mortality is still unknown and will need to be verified in larger and adequately powered studies

In addition to this, we were also intrigued to know whether the application of our preconditioning stimulus had a different impact based on the different technique of myocardial preservation, and particularly based on the administration of cold blood antegrade cardioplegia versus warm antegrade/retrograde cardioplegia: amongst preconditioned CABG patients, 27 received antegrade cardioplegia, 14 ICCF and 16 antegrade/retrograde cardioplegia (**Table 4.1)**. We found that only the application of RIPC in the context of ICCF significantly reduced hsTnT release, whereas PMI magnitude in control and preconditioned patients undergoing antegrade/retrograde

cardioplegia was essentially similar. This therefore suggests that the protective effect of RIPC might not be significant in patients receiving this technique and this could well be related to either the relatively small PMI magnitude achieved in these subjects or to the relatively small level of additional benefit provided by limb IR. Once again it is important to emphasise that the current study was not powered for this type of analysis and therefore findings arising from here should be taken with very careful consideration: only large randomised RCTs will be able to further clarify firstly the role of antegrade/retrograde cardioplegia in myocardial protection in patients undergoing cardiac surgery, and secondly whether the combination of RIPC with this technique might be able to provide further beneficial effects on PMI and clinical outcomes. In this regard, our ERICCA trial, although not originally powered for this evaluation as the above findings were only obtained following ERICCA initiation, will hopefully be able to further clarify these crucial aspects.

Table 4.6. Total AUC in patients undergoing elective CABG surgery with antegrade/retrograde cardioplegia, antegrade cardioplegia or ICCF

Technique of myocardial preservation Intervention (n)	**CABG**			
	Control (mean (SD))	**RIPC (mean (SD))**	**Difference (95% CI)**	**P value**
Antegrade Cardioplegia Control: n=28 RIPC: n=27	34.30 (20.35)	28.19 (13.55)	6.11 (-3.33, 15.55)	0.200
Antegrade/retrograde Cardioplegia Control: n=10 RIPC: n=16	17.76 (7.54)	17.88 (9.34)	-0.12 (-7.37, 7.12)	0.973
Intermittent cross-clamp fibrillation Control: n=16 RIPC: n=11	32.88 (18.77)	20.69 (6.04)	12.19 (0.66, 24.32)	0.049

CABG=coronary artery bypass graft; CI=confidence interval; RIPC=remote ischaemic preconditioning

CHAPTER 5

5. Effect of multi-limb RIPC on cardioprotection in patients undergoing cardiac bypass surgery using GTN

5.1. Introduction

We have so far demonstrated that an enhanced preconditioning stimulus reduces PMI in an unselected cohort of patients undergoing elective cardiac surgery and irrespective of the technique of myocardial preservation, and in patients having CABG with or without valve surgery. During these analyses, we found no significant difference in baseline and surgical parameters between preconditioned and control patients in the vast majority of cases. However, we identified in the peri-operative administration of iv GTN one of the most important variables with different frequency between the intervention groups **(Table 5.1)**: GTN is a nitrate functioning as a NO donor and is widely used in the context of cardiac surgery and particularly CABG surgery in order to achieve effective and rapid BP control and ensure coronary vasodilatation, thereby improving intra-operative coronary perfusion and maintaining graft patency post-operatively (509). NO has been demonstrated to interfere with IPC and RIPC in experimental studies, however its role in the clinical setting is yet to be clarified (510-521). We have therefore conducted a further retrospective analysis in order to determine whether the intra-operative use of GTN has an impact on the protective effects of RIPC on PMI in patients undergoing cardiac surgery.

Table 5.1. Total AUC reduction and GTN use in different subgroup analyses

Operation	Control		RIPC		AUC P value	GTN given P value
	AUC (µg/L) (mean (sd))	GTN given (N, %)	AUC µg/L) (mean (sd))	GTN given (N, %)		
All cardiac surgery	36.307 (24.542)	65/89 (73%)	27.004 (16.523)	53/89 (60%)	0.004	0.035
All cardiac surgery Cardioplegia	37.089 (25.730)	51/73 (70%)	27.942 (17.386)	41/75 (55%)	0.014	0.037
All cardiac surgery Cross-clamp fibrillation	32.885 (18.771)	14/16 (88%)	20.692 (6.039)	12/14 (86%)	0.049	0.886
CABG+/-valve surgery	33.526 (20.164)	51/64 (80%)	24.772 (12.640)	47/66 (71%)	0.004	0.140
CABG alone	30.753 (18.948)	46/54 (85%)	23.609 (12.004)	41/57 (72%)	0.022	0.045
CABG alone Cardioplegia	29.832 (19.206)	32/38 (84%)	24.355 (13.052)	29/43 (67%)	0.147	0.053
CABG/AVR	48.22 (21.01)	5/10 (50%)	31.75 (14.82)	6/9 (67%)	0.068	0.629
Valve surgery alone	43.925 (33.144)	14/25 (56%)	33.395 (23.719)	6/23 (26%)	0.229	0.450
AVR	38.499 (37.661)	7/15 (47%)	27.947 (24.678)	2/14 (14%)	0.402	0.060
MV surgery	53.246 (25.406)	6/9 (67%)	44.902 (19.314)	3/8 (38%)	0.472	0.229

AUC=area-under-the-curve; RIPC=remote ischaemic preconditioning; GTN=glyceryl trinitrate; sd=standard deviation; CABG=coronary artery bypass graft; AVR=aortic valve replacement; MV=mitral valve.

5.2. Results

Of the 178 patients included in our principal study, 3 were excluded from this sub-group analysis as there was no clear documentation in their medical notes on whether iv GTN had been used during surgery: of the remaining 175 subjects, 118 received GTN intra-operatively and were randomised to control (n=65) or RIPC (n=53),

56 only patients were not administered GTN, of which 21 received the sham protocol and 35 the RIPC protocol **(Table 5.1)**. Amongst patients receiving GTN we found no statistically significant difference of baseline characteristics between control and RIPC subjects, whereas in the group not receiving GTN preconditioned patients presented a lower NYHA status and incidence of hypercholesterolemia **(Table 5.2).**

Additionally, there was no statistically significant difference of surgical procedure parameters between control and preconditioned patients in either the GTN or no-GTN groups **(Table 5.3)**. Additive EuroSCORE and use of anaesthetic regimes were comparable between groups although subjects not receiving GTN had a lower proportion of patients undergoing CABG alone and a higher proportion of patients undergoing AVR compared to subjects receiving GTN **(Table 5.3)**.

In the GTN group, mean hsTnT concentrations were lower in preconditioned patients at all the post-operative time-points (**Table 5.4, Fig. 5.1**), however, with a statistical significance only at 72 hours: RIPC reduced total AUC from 30.81±17.56μg/L to 26.69±13.93μg/L [4.12; CI -1.92, 10.17; p=0.179], which corresponded to only a non-significant reduction of 13% (**Table 5.4, Fig. 5.3**). Conversely, in patients not administered GTN, the RIPC group had significantly lower mean hsTnT levels at all the post-operative time-points and a decreased total AUC from 50.52±34.20 μg/L to 27.86±20.01 μg/L, which corresponded to a very significant reduction of 45% [22.66; CI 8.03, 37.29; p=0.003] **(Table 5.4, Figs. 5.2, 5.4)**.

With regards to secondary endpoints, the use of combined GTN and RIPC was associated with a significantly improved urine output at 24 and 72 hours post-operatively and as a total amount over the three days post-surgery, whereas no significant difference was found in the remaining end-points **(Table 5.5)**.

Table 5.2. Baseline characteristics of patients in GTN and No-GTN groups

Patients	GTN (mean (SD))			No-GTN (mean (SD))		
	Control (n=65)	RIPC (n=53)	P value	Control (n=21)	RIPC (n=35)	P value
Age	66±9	64±10	0.473	68±12	65±11	0.255
Gender			0.108			0.747
Male	48 (73.8%)	46 (86.8%)		17 (81.0%)	26 (74.3%)	
Female	17 (26.2%)	7 (13.2%)		4 (19.0%)	9 (25.7%)	
Ethnicity			0.607			0.984
Caucasian	54 (83.1%)	41 (77.4%)		17 (81.0%)	29 (77.4%)	
Asian	8 (12.3%)	9 (17.0%)		2 (9.5%)	3 (8.6%)	
Afro-Caribbean	2 (3.1%)	3 (5.7%)		2 (9.5%)	3 (8.6%)	
Chinese	1 (1.5%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
BMI	28.5±5.3	29.5±8.3	0.457	28.6±6.2	27.8±4.7	0.615
SBP *(mmHg)*	128.32±18.2	131.1±16.0	0.392	135.1±18.2	126.1±15.0	0.052
DBP *(mmHg)*	70.3±9.0	71.56±8.96	0.441	72.2±9.7	69.9±10.14	0.393
HR *(bpm)*	69.8±11.9	66.5±10.1	0.108	68.5±11.0	65.8±9.4	0.336
Smoking History			0.589			0.740
Smoker	8 (12.3%)	9 (17.0%)		2 (9.5%)	2 (5.7%)	
Ex-smoker	39 (60.0%)	27 (50.9%)		13 (61.9%)	13 (37.1%)	
Non-smoker	18 (27.7%)	17 (32.1%)		6 (28.6%)	20 (57.1%)	
Family History of IHD	45 (69.2%)	38 (71.7%)	0.841	11 (52.4%)	6 (74.3%)	0.145
NYHA Class	2.61±0.9	2.56±0.76	0.749	3.24±0.6	2.56±0.76	0.001
CCS Class	2.19±1.14	2.46±1.07	0.210	2.10±1.09	2.18±1.11	0.792
LVEF			0.247			0.197
>50%	47 (72.3%)	39 (73.6%)		20 (95.2%)	27 (77.1%)	
30%-50%	16 (24.6%)	9 (17.0%)		1 (4.8%)	7 (20.0%)	
<30%	2 (3.0%)	5 (9.4%)		0 (0.0%)	1 (1.9%)	
Co-morbidities						
Diabetes Mellitus	19 (29.2%)	18 (34.0%)	0.690	5 (23.8%)	10 (28.6%)	0.764
Hypertension	50 (76.9%)	36 (67.9%)	0.188	17 (81.0%)	29 (82.9%)	1.000
Hypercholesterolemia	50 (76.9%)	37 (69.8%)	0.407	12 (57.1%)	31 (88.6%)	0.010
Atrial Fibrillation	13 (20.0%)	7 (13.2%)	0.303	4 (19.0%)	5 (14.3%)	0.715
Previous MI	19 (29.2%)	20 (37.7%)	0.432	4 (19.0%)	8 (22.9%)	0.432
Previous PCI	9 (13.8%)	7 (13.2%)	1.000	2 (9.5%)	8 (22.9%)	1.000
Previous CVA/TIA	8 (12.3%)	3 (5.7%)	0.657	1 (4.8%)	2 (5.7%)	1.000
Previous Cardiac Surgery	2 (3.0%)	1 (1.9%)	0.794	0 (0.0%)	1 (1.9%)	0.794
Other comorbidities	6 (9.4%)	1 (1.9%)	0.245	6 (28.6%)	3 (8.1%)	0.386
Peripheral Arterial Disease	3 (4.6%)	0 (0.0%)	0.251	3 (14.3%)	1 (2.9%)	0.143
Drug History						
Aspirin	48 (76.2%)	46 (90.2%)	0.148	16 (76.2%)	25 (66.0%)	0.578
Clopidogrel/Prasugrel	23 (60.0%)	18 (36.0%)	0.355	2 (9.2%)	6 (17.6%)	0.182
Warfarin	9 (14.2%)	2 (3.9%)	0.052	0 (0.0%)	1 (2.9%)	0.264
Beta-blocker	44 (69.9%)	35 (68.6%)	0.662	10 (47.6%)	22 (64.7%)	0.266
Calcium Channel Blocker	23 (60.0%)	7 (21.6%)	0.179	8 (38.1%)	11 (32.4%)	0.186
Statin	54 (84.7%)	43 (84.3%)	0.394	15 (71.4%)	28 (82.4%)	0.394
ACE-I/ARB	41 (65.1%)	31 (60.8%)	0.914	14 (66.7%)	23 (67.6%)	0.239
Long acting nitrates	14 (19.0%)	9 (17.6%)	0.423	0 (0.0%)	3 (8.8%)	0.371
Antidiabetics						
Insulin	6 (9.5%)	5 (9.8%)	0.631	1 (4.8%)	3 (8.8%)	1.000
Biguanide	2 (3.2%)	2 (3.9%)	0.930	1 (3.2%)	3 (8.8%)	0.812
Sulphonylurea	8 (12.7%)	4 (7.8%)	0.635	1 (4.8%)	2 (5.9%)	1.000
Diuretics	20 (31.7%)	19 (37.3%)	0.769	6 (28.6%)	11 (32.40%)	0.611

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA= Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF=left ventricular ejection fraction.

Table 5.3. Details of surgical procedure of patients in GTN and No-GTN groups

Patients	GTN			No-GTN		
	Control (n=65)	RIPC (n=53)	P value	Control (n=21)	RIPC (n=35)	P value
Indication for Surgery			0.206			0.159
Angina	35 (53.8%)	27 (50.9%)		6 (28.6.8%)	12 (34.3%)	
MI	11 (16.9%)	14 26.4%)		1 (4.8%)	5 (14.3%)	
Valve Disease	13 (20.0%)	5 (9.4%)		10 (47.6%)	18 (51.4%)	
Angina and Valve Disease	6 (9.2%)	2 (3.8%)		0 (0.0%)	0 (0.0%)	
MI and Valve Disease	0 (0.0%)	1 (1.9%)		1 (4.8%)	0 (0.0%)	
SBE						
EuroSCORE	3.54±1.79	3.49±2.67	0.908	4.52±2.54	4.00±2.50	0.454
Additive perioperative risk			0.333			0.901
Low (EuroSCORE 0-2)	20 (30.8%)	18 (34.0%)		5 (23.8%)	10 (28.6%)	
Medium (EuroSCORE 3-5)	14 (55.4%)	23 (43.4%)		9 (42.9%)	15 (43.4%)	
High (EuroSCORE >5)	9 (13.8%)	12 (22.6%)		7 (33.3%)	10 (28.6%)	
Bypass-time (min)	103.76±29.95	88.32±31.99	0.176	103.76±29.95	88.32±31.99	0.704
Cross-clamp time (min)	63.52±27.38	59.46±19.28	0.357	65.85±23.04	65.14±35.59	0.937
Cardioprotection			0.645			0.626
Blood cardioplegia	51 (78.5%)	41 (77.4%)		19 (90.5%)	33 (94.3%)	
Cross-clamp fibrillation	14 (21.5%)	12 (22.6%)		2 (9.5%)	2 (5.7%)	
Number of grafts			0.374			0.415
Zero	14 (21.5%)	6 (11.3%)		11 (52.4%)	17 (48.6%)	
One	2 (3.1%)	4 (7.5%)		2 (9.5%)	1 (2.9%)	
Two	15 (23.1%)	10 (18.9%)		4 (19.0%)	4 (11.4%)	
Three	23 (35.4%)	25 (47.2%)		4 (19.0%)	10 (28.6%)	
Four	11 (16.9%)	8 (15.1%)		0 (0.0%)	3 (8.6%)	
Operation			0.304			0.386
CABG	46 (70.8%)	41 (77.4%)		6 (28.6%)	15 (42.9%)	
CABG/AVR	5 (7.7%)	6 (11.3%)		4 (19.0%)	3 (8.6%)	
AVR	12 (18.5%)	8 (15.1%)		12 (57.1%)	15 (42.9%)	
MV surgery	6 (9.2%)	3 (5.7%)		3 (9.2%)	5 (14.3%)	
AVR/MVR	1 (1.5%)	1 (1.9%)		0 (0.0%)	0 (0.0%)	
Anesthetic agents						
Induction						
Anti-nicotinic agents			0.544			0.185
Rocuronium	48 (80.0%)	45 (90.0%)		17 (81.0%)	30 (88.2%)	
Pancuronium	10 (16.7%)	4 (8.0%)		4 (19.0%)	2 (5.9%)	
Vecuronium	2 (3.3%)	1 (2.0%)		0 (0.0%)	2 (5.9%)	
Midazolam	34 (56.7%)	22 (44.0%)	0.250	11 (52.4%)	11 (22.4%)	0.166
Etomidate	7 (11.3%)	6 (12.0%)	1.000	1 (4.8%)	1 (2.9%)	1.000
Fentanyl	63 (100%)	54 (100%)	1.000	21 (100%)	34 (100%)	1.000
Propofol	56 (90.3%)	43 (86.0%)	0.559	17 (81.0%)	33 (97.1%)	0.064
Maintenance						
Propofol	63 (100%)	54 (100%)	1.000	21 (100%)	34 (100%)	1.000
Volatile Anesthetics			0.458			0.519
Isoflurane	57 (91.9%)	48 (96.0%)		21 (100%)	32 (94.1%)	
Sevoflurane	5 (8.1%)	2 (4.0%)		0 (0.0%)	2 (5.9%)	

RIPC=Remote Ischemic Preconditioning; CABG=Coronary artery bypass graft; AVR=Aortic valve replacement.

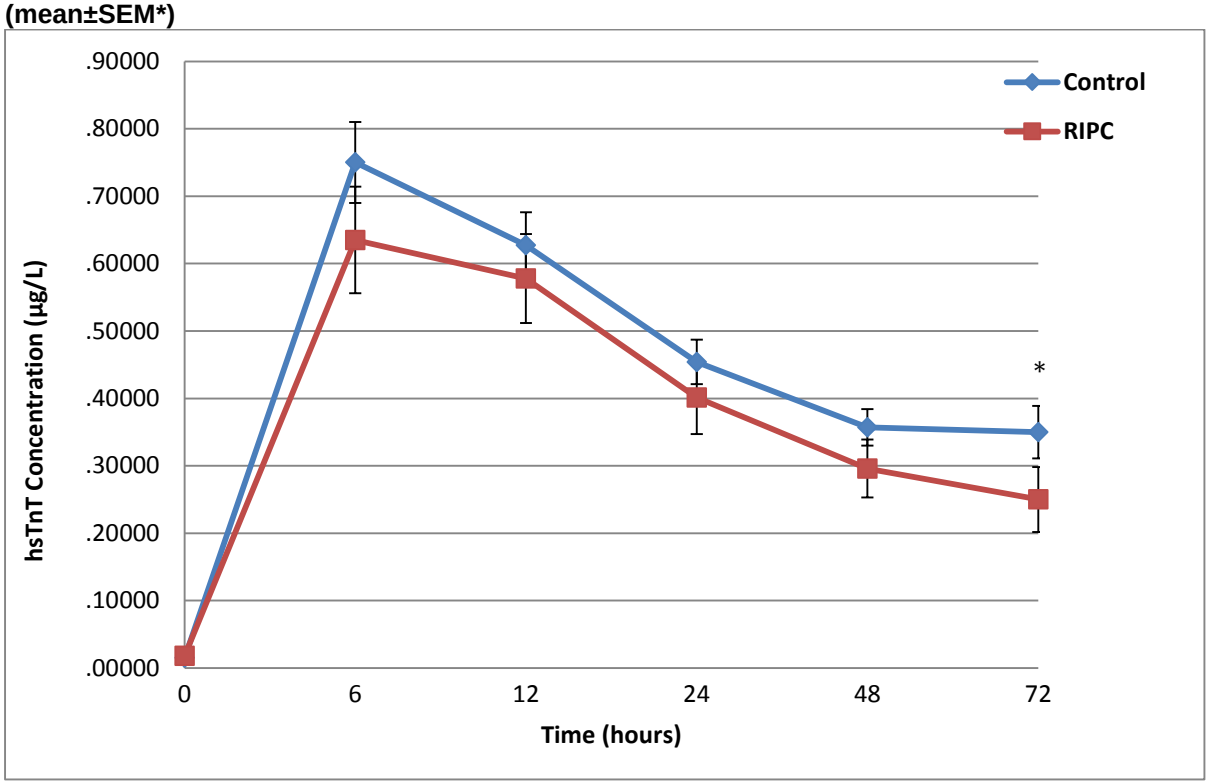
Table 5.4. Mean hsTnT and AUC in patients in GTN and no-GTN groups

Endpoint	GTN intra-operatively	Control GTN: n=65 No-GTN: n=53 (mean (sd))	RIPC (n=53) GTN: n=21 No-GTN: n=35 (mean (sd))	Difference (95% CI)	P value
hsTnT *(µg/L)*					
Pre- operatively	GTN	0.015 (0.015)	0.018 (0.023)	-0.002 (-0.009, 0.004)	0.510
	No-GTN	0.027 (0.027)	0.011 (0.015)	0.016 (0.005, 0.027)	0.018
6 hours post-operatively	GTN	0.750 (0.487)	0.635 (0.319)	0.114 (-0.039, 0.268)	0.129
	No-GTN	0.905 (0.468)	0.591 (0.464)	0.312 (0.055, 0.570)	0.018
12 hours post-operatively	GTN	0.627 (0.395)	0.578 (0.371)	0.050 (-0.091, 0.190)	0.485
	No-GTN	0.905 (0.447)	0.533 (0.389)	0.372 (0.114, 0.144)	0.002
24 hours post-operatively	GTN	0.454 (0.265)	0.401 (0.239)	0.054 (-0.039, 0.147)	0.254
	No-GTN	0.745 (0.446)	0.320 (0.317)	0.320 (0.093, 0.546)	0.007
48 hours post-operatively	GTN	0.358 (0.214)	0.296 (0.168)	0.061 (-0.011, 0.133)	0.096
	No-GTN	0.701 (0.678)	0.327 (0.249)	0.374 (0.056, 0.693)	0.023
72 hours post-operatively	GTN	0.350 (0.300)	0.250 (0.165)	0.100 (0.007, 0.194)	0.036
	No-GTN	0.552 (0.436)	0.321 (0.281)	0.231 (0.037, 0.424)	0.020
Total 72 hours AUC	GTN	30.81 (17.56)	26.69 (13.93)	4.12 (-1.92, 10.17)	0.179
	No-GTN	50.52 (34.20)	27.86 (20.01)	22.66 (8.03, 37.29)	0.003

RIPC=Remote ischemic preconditioning; SD=standard deviation; CI=confidence interval; hsTnT=high sensitivity Troponin T; AUC= area-under-the-curve; GTN=glyceryl trinitrate

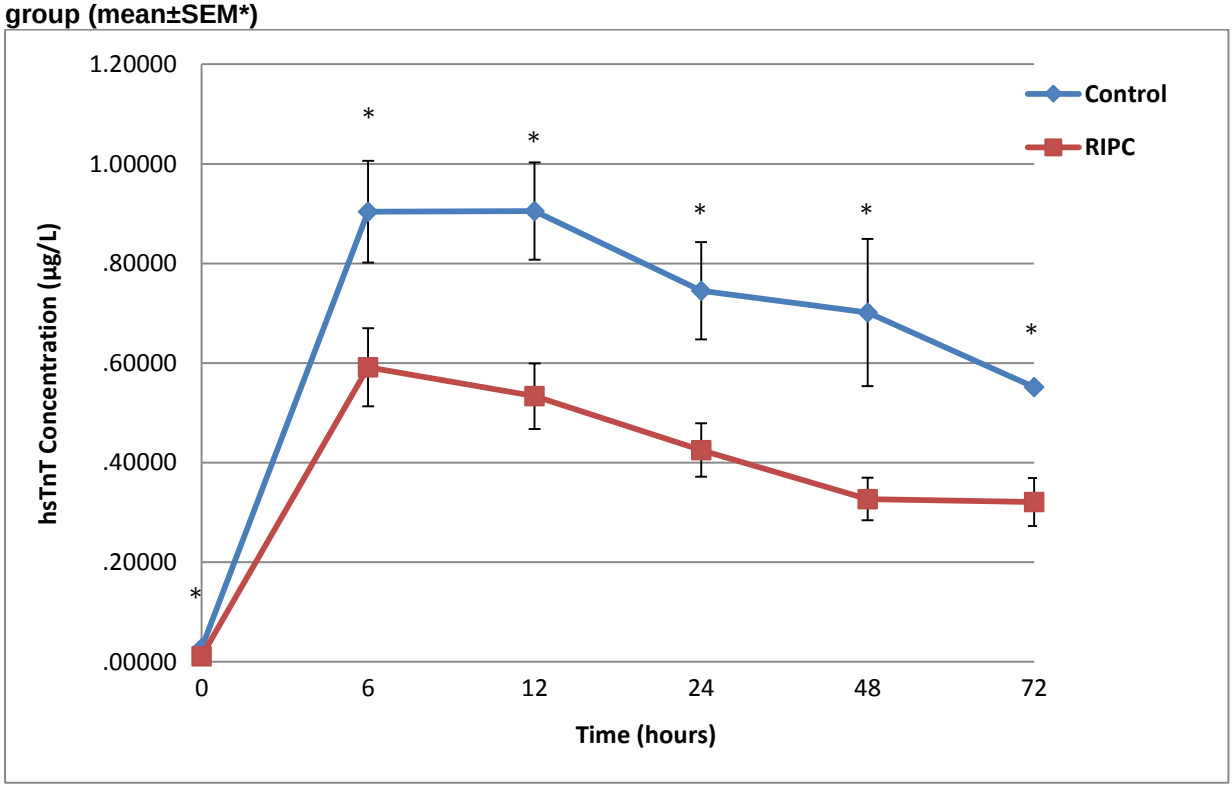
In patients not receiving GTN, interestingly RIPC led to a significantly lower incidence of post-operative AKI with 4 new cases vs 14 new cases in the control group and therefore to a reduction of 71% of cases (p=0.042) **(Table 5.6)**: remarkably, 5 deaths occurred in the sham group and none in the RIPC group and this was close to statistical significance (p=0.061). We did not attempt to conduct further subgroup analyses within the GTN and no-GTN groups with regards to type of operation and/or technique of myocardial preservation, as this would have led to small sample size with therefore unreliable tests results.

Fig. 5.1. Mean high-sensitivity Troponin T levels at 0, 6, 12, 24, 48 and 72 hours in the GTN group (mean±SEM*)



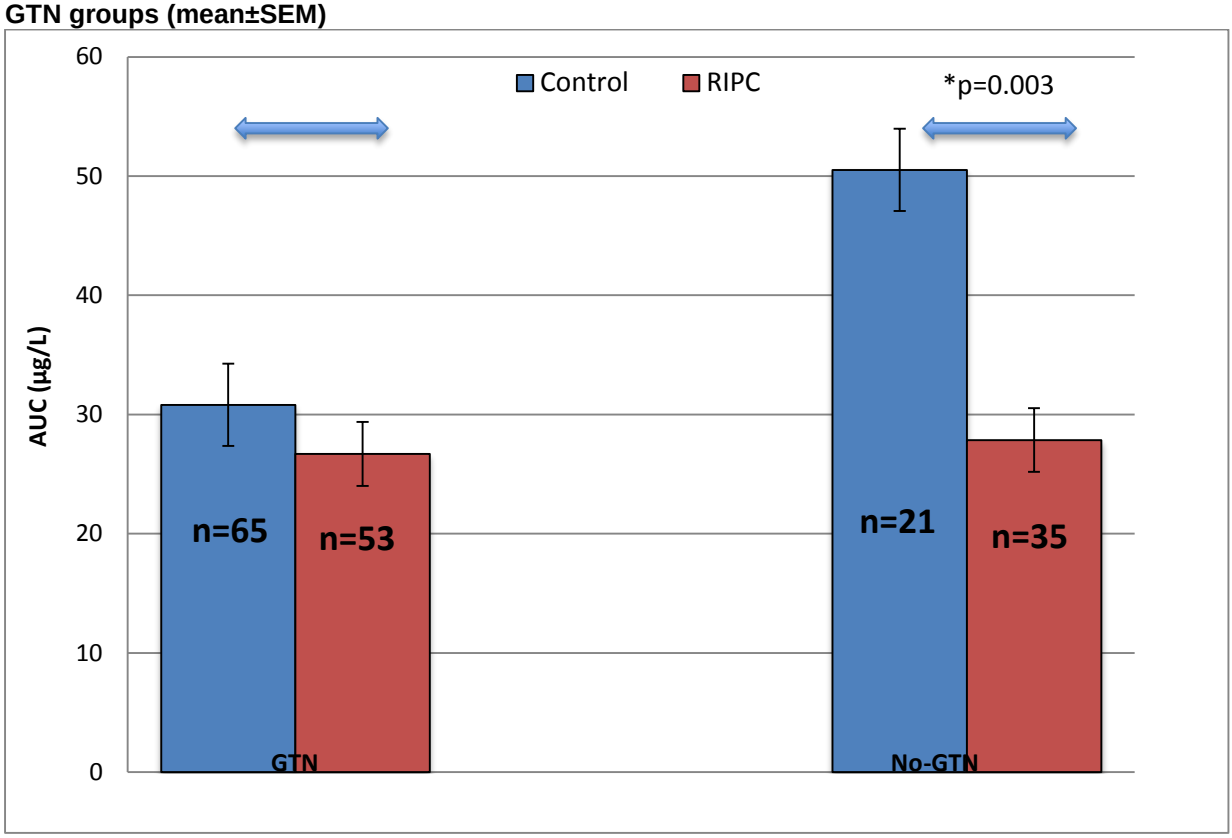
RIPC=remote ischaemic preconditioning; hsTnT=high sensitivity troponin-T; SEM=standard error of the mean. * Unpaired Student T-Test

Fig. 5.2. Mean high-sensitivity Troponin T levels at 0, 6, 12, 24, 48 and 72 hours in the No-GTN group (mean±SEM*)



RIPC=remote ischaemic preconditioning; hsTnT=high sensitivity troponin-T; SEM=standard error of the mean. *p<0.05 (unpaired Student T-Test)

Fig. 5.3. Total Area under the Curve of high-sensitivity Troponin T in patients in GTN and No-GTN groups (mean±SEM)



RIPC=remote ischaemic preconditioning; AUC=area under the curve; SEM=standard error of the mean
*Unpaired Student T-Test

Table 5.5. Summary of major secondary endpoints in the GTN group*

Endpoint	Control (n=65) (mean (sd))	RIPC (n=53) (mean (sd))	Difference (95% CI)	P value
CK (*μg/L*)				
Total AUC	33021.79 (23496.79)	37912.06 (27494.08)	-4890.27 (-16877.14, 7096.60)	0.419
Creatinine (mg/ml)				
Pre-operatively	86.97 (19.12)	88.04 (29.37)	-1.07 (-9.96, 7.82)	0.812
24 hours post-operatively	91.71 (27.08)	88.74 (25.87)	2.97 (-6.76, 12.70)	0.546
48 hours post-operatively	102.89 (46.11)	93.00 (38.29)	9.82 (-5.79, 25.57)	0.214
72 hours post-operatively	99.25 (52.97)	92.04 (40.35)	7.21 (-10.29, 24.70)	0.416
Urine Output (ml)				
24 hours post-operatively	1900.3 (633.6)	2207.7 (691.9)	-307.46 (-560.4, 554.5)	0.018
48 hours post-operatively	2169.2 (908.2)	2333.9 (861.6)	-164.8 (-528.2, 198.6)	0.370
72 hours post-operatively	1922.1 (801.5)	2490.3 (900.1)	-568.2 (-977.1, -159.3)	0.007
Total	5790.2 (1834.5)	6706.4 (1618.3)	-916.2 (-1745.9, -86.4)	0.031
AKI score				
0	16	30		
1	3	2		
2	2	2		
3	0	1		
Acute Kidney Injury	5	5		0.476
Inotrope score				
Post bypass	7.09 (14.46)	6.99 (16.45)	0.094 (-5.67, 5.86)	0.974
24 hours post-operatively	11.26 (22.13)	10.74 (18.14)	0.514 (-7.16, 8.19)	0.895
48 hours post-operatively	7.40 (18.10)	6.74 (16.15)	6.53 (-5.85, 7.16)	0.843
72 hours post-operatively	4.08 (14.92)	2.43 (10.56)	1.65 (-3.30, 6.59)	0.510
Total	30.28 (61.45)	25.70 (46.63)	4.59 (-16.23, 25.40)	0.663
New onset AF	3	3		0.661
Length of ICU stay (days)	2.0 (2.0-4.0)**	2.0 (1.0-4.0)**		0.256***
Length of hospital stay (days)	9.0 (7.0-13.0)**	8.0 (6.0-9.0)**		0.068***
Clinical outcomes at six weeks				
Death	0	0		1.000
Myocardial infarction	0	0		1.000
Stroke	0	1		0.417
Revascularization	0	0		1.000

Table 5.6. Summary of major secondary endpoints in the No-GTN group*

Endpoint	Control (n=21) (mean (sd))	RIPC (n=35) (mean (sd))	Difference (95% CI)	P value
CK (*μg/L*)				
Total AUC	32194.11 (102249.05)	34616.80 (27434.32)	-2422.69 (-13605.14, 8759.90)	0.664
Creatinine (mg/ml)				
Pre-operatively	87.38 (22.23)	83.49 (24.14)	3.90 (-9.08, 16.87)	0.550
24 hours post-operatively	97.76 (38.74)	86.57 (28.73)	11.19 (-6.95, 29.34)	0.222
48 hours post-operatively	110.33 (59.93)	91.17 (39.78)	19.16 (-7.53, 45.87)	0.156
72 hours post-operatively	102.90 (54.60)	90.91 (47.63)	11.99 (-15.86, 39.84)	0.392
Urine Output (ml)				
24 hours post-operatively	2226.9 (1039.7)	2116.3 (517.9)	110.60 (-316.3, 537.5)	0.605
48 hours post-operatively	2075.6 (994.9)	2373.2 (843.6)	-297.6 (-823.8, 228.5)	0.261
72 hours post-operatively	2318.5 (1022.2)	2479.7 (739.7)	-161.2 (-868.7, 546.3)	0.604
Total	5988.5 (1578.8)	6706.4 (1559.9)	-718.9 (-1875.1, 437.4)	0.214
AKI score				
0	51	49		
1	8	4		
2	3	0		
3	3	0		
Acute Kidney Injury	14	4		0.042
Inotrope score				
Post bypass	5.72 (10.92)	6.60 (13.52)	-0.856 (-7.87, 6.16)	0.808
24 hours post-operatively	12.55 (18.05)	7.42 (13.86)	5.12 (-3.54, 13.79)	0.241
48 hours post-operatively	11.83 (22.28)	3.68 (10.28)	8.16 (-0.70, 17.02)	0.127
72 hours post-operatively	10.25 (21.37)	0.54 (2.26)	9.71 (-0.04, 19.47)	0.051
Total	39.80 (52.85)	18.12 (34.97)	21.68 (-4.83, 48.20)	0.105
New onset AF	18	7		0.071
Length of ICU stay (days)	3.0 (2.0-5.0)**	2.0 (1.0-3.0)**		0.767***
Length of hospital stay (days)	8.5 (7.0-11.5)**	8.0 (6.0-10.5)**		0.485***
Clinical outcomes at six weeks				
Death	5	0		0.061
Myocardial infarction	1	0		0.643
Stroke	0	0		1.000
Revascularization	0	0		1.000

*List of abbreviations
RIPC=Remote ischemic preconditioning; CK=Creatinine Kinase; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve
**Results shown as median (inter-quartile range); *** P-value for Mann-Whitney-Wilcoxon test

5.3. Discussion

With this subgroup analysis we have demonstrated that the application of our enhanced preconditioning stimulus, in the absence of intra-operative GTN administration, leads to a significant reduction of the mean hsTnT release at all the specified time-points and more importantly of the total hsTnT release over the 3 post-operative days, with a 45% reduction of AUC, compared to control patients who did not received nitrates during cardiac surgery. Conversely, in patients administered GTN, we found no statistically significant difference of the 72 hours hsTnT AUC between the two intervention groups. This extremely intriguing finding suggests a potential involvement of NO donors in the RIPC mechanisms and particularly that the beneficial effects induced by RIPC could be inhibited by the administration of NO donors.

It is well established that NO improves LV function and oxygen demand/supply ratio at low concentration in short-term hibernating myocardium (510), whereas it triggers inflammation and reduces LV systolic function at high concentration (512). NO has been demonstrated to play a major role in the initiation of the late phase of IPC (511, 513, 514): in particular, in conscious rabbits subjected to 30-minute coronary occlusion and 3 days of reperfusion, iv administration of nitroglycerin reduced infarct size when it was delivered 1 hour prior to occlusion but also interestingly when the interval between nitroglycerin infusion and occlusion was extended to 24 and 72 hours, thereby indicating a potent late preconditioning effect (515). Preclinical studies demonstrated that endothelial NO-synthase (eNOS) is a potent trigger of delayed preconditioning whilst inducible NO-synthase (iNOS) functions as a mediator of delayed preconditioning (516): iNOS overexpression has been associated with inhibition of mPTP opening (517) and additionally NO has also been found to be a

potent mediator of IPost (518). More recently, pre-treatment with the NO-donor S-nitroso-N-acetylpenicillamine or nerve transection have been demonstrated to abolish the cardioprotective effect of intra-arterial adenosine and RIPC in rabbits (519).

Within the clinical setting, in a post-hoc analysis examining the late preconditioning mimetic effects of nitroglycerin on PMI in patients undergoing PCI for single obstructive CAD (520), no significant difference in PMI was found in patients receiving nitroglycerin prior to preconditioning and PCI. Interestingly, in the study by Wagner and colleagues (295), there was a small, significant increase in iNOS expression after CPB in the control group, whereas no significant difference was found before or after CPB in eNOS concentration in the same group or iNOS or eNOS levels in the RIPC or tramadol patients. Crucially, in a context very similar to our study, Kleinbongard and colleagues (521) found that the administration of nitroglycerin after induction of anaesthesia did not impact on final PMI magnitude in either preconditioned or control patients. In addition, in the largest proof-of-concept study on RIPC in cardiac surgery at the time of our recruitment (286), patients routinely received iv GTN administration in the peri-operative period, which however, has not always been clearly documented in similar works.

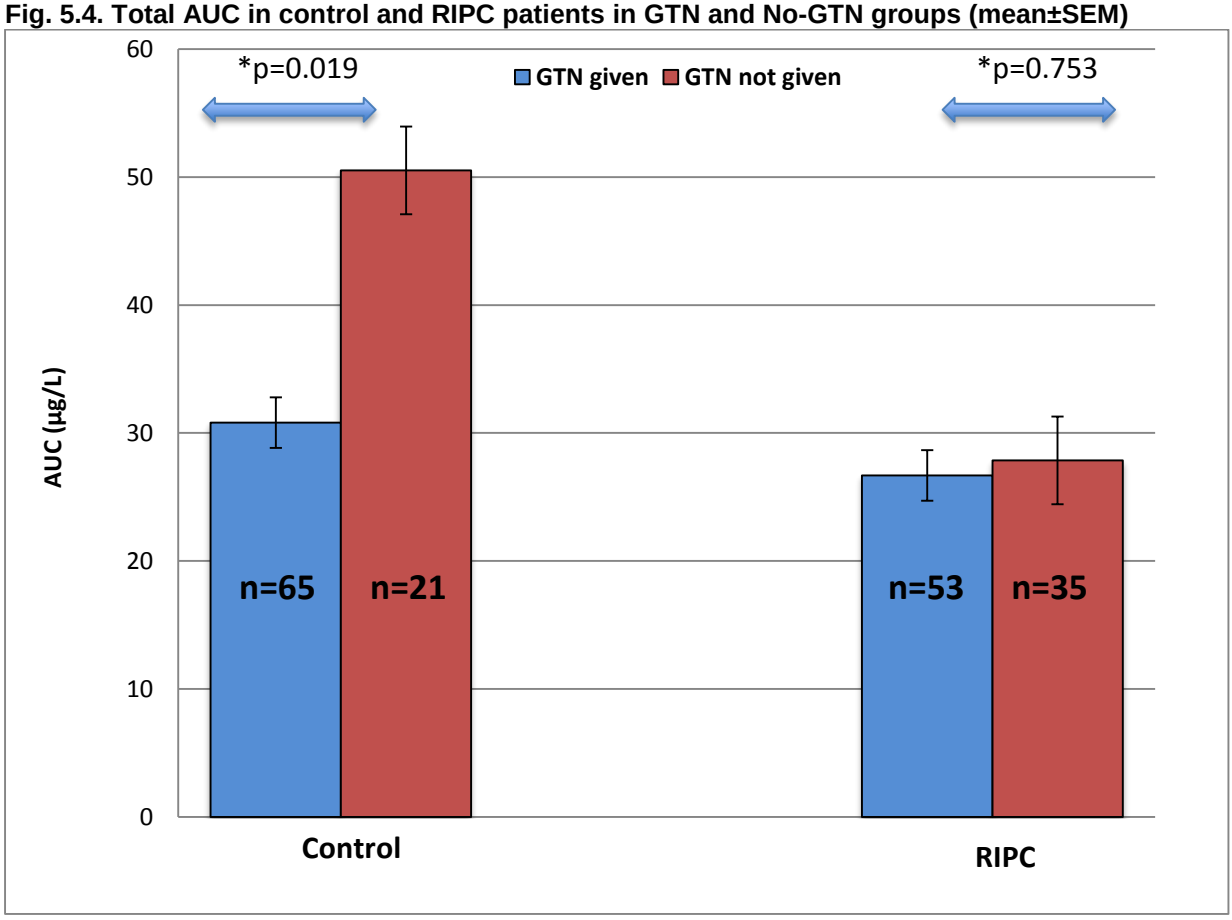
Whether our potentially crucial finding might give an explanation to the failure to observe RIPC cardioprotection in these RTCs is clearly difficult to establish but nevertheless it offers an important suggestion for future studies: this is therefore the first analysis to demonstrate a significant impact of iv nitrates in patients undergoing elective cardiac surgery.

Furthermore, our control GTN-subgroup sustained 39% less PMI than the control no-GTN subgroup, with a total AUC of 30.81±17.56 μg/L versus 50.52±34.20 μg/L [-19.70; CI -35.79, 3.61; p=0.019] **(Table 5.7, Fig. 5.5)**. Amongst RIPC patients,

we found no significant difference in total AUC between GTN and no-GTN subjects, with AUCs of 26.69±13.93 µg/L and 27.86±20.01 µg/L respectively [-1.17; CI -8.53, 6.19; p=0.753] **(Table 5.7, Fig. 5.5)**. There are two potential explanations to our findings:

1. the beneficial effects of RIPC on PMI are inhibited by GTN, and therefore RIPC may not be able to elicit cardioprotection in the presence of concomitant iv GTN administration ;
2. GTN is able to enhance cardioprotection and the additional protection provided by RIPC may not be significant.

Fig. 5.4. Total AUC in control and RIPC patients in GTN and No-GTN groups (mean±SEM)



GTN=glycerine trinitrate, AUC=area-under-the-curve; RIPC=remote ischaemic preconditioning; SEM=standard error of the mean. * Unpaired Student T-Test

Table 5.7. Major details of surgery and total AUC in patients undergoing elective cardiac surgery in GTN and No-GTN groups

Parameters		GTN Control: n=65 RIPC: n=53 (mean (sd))	No-GTN Control: n=21 RIPC: n=35 (mean (sd))	Difference (95% CI)	P value
EuroSCORE	Sham	3.54 (1.79)	4.52 (2.54)	-0.99 (-2.21, 0.24)	0.111
	RIPC	3.49 (2.68)	4.00 (2.50)	-0.51 (-1.64, 0.620)	0.372
Cardio-pulmonary bypass time	Sham	95.84 (33.08)	95.57 (30.20)	0.270 (-15.97, 16.51)	0.974
	RIPC	88.98 (20.37)	91.51 (42.56)	-2.53 (-16.09, 11.02)	0.745
Cross-clamp time	Sham	63.52 (27.38)	65.85 (23.04)	-2.33 (-15.85, 11.18)	0.732
	RIPC	59.46 (19.25)	65.14 (35.59)	-5.90 (-5.68, 6.06)	0.392
AUC	Sham	30.81 (17.56)	50.52 (34.20)	-19.70 (-35.79, 3.61)	0.019
	RIPC	26.69 (13.93)	27.86 (20.01)	-1.17 (-8.53, 6.19)	0.753

GTN=glycerine trinitrate, CI=confidence interval; sd=standard deviation; AUC=area-under-the-curve

In addition, we have found an extremely intriguing association between total hsTnT release and GTN use between preconditioned and control patients in the vast majority of cases. Crucially, we observed a significantly higher use of intra-operative GTN in control patients undergoing cardiac surgery, cardiac surgery with cardioplegia and CABG surgery alone (p=0.035, 0.037 and 0.045 respectively), which conversely corresponded to a significantly lower PMI in preconditioned patients (p=0.004, 0.014 and 0.022 respectively) **(Table 5.7)**. Interestingly, in patients undergoing CABG surgery alone with cardioplegia, CABG plus AVR, all valve surgery alone, AVR alone and MV surgery alone, where there was no statistically significant difference between control and RIPC subjects with regards to GTN administration (p=0.053, 0.629, 0.450, 0.060 and 0.229 respectively), we also found no statistically significant difference in total hsTnT AUC (p=0.147, 0.068, 0.229, 0.402 and 0.472 respectively). Only in

patients receiving ICCF or undergoing CABG ±valve surgery, we found a discrepancy between a non-significant difference of GTN use (p=0.886 and 0.140 respectively) and a significant PMI reduction in RIPC subjects (p=0.049 and 0.004 respectively).

Moreover, whilst in GTN-administered patients the incidence of AKI and new AF onset was identical between preconditioned and control groups, in the no-GTN group RIPC reduced AKI incidence by 71% with 14 new cases in the control group and 4 new cases in the RIPC group (p=0.041): this represents the first evidence that our enhanced preconditioning stimulus achieved significant reno-protection in patients undergoing cardiac surgery. Whether GTN plays an important role in reducing renal IRI is difficult to establish at this stage but certainly we provide an intriguing suggestion for future studies in this field.

Nevertheless it is also important to note that whilst the number of patients was sufficiently high in the GTN group (65 control and 53 preconditioned), with no significant hsTnT reduction found, the sample size was considerably lower in the no-GTN group (21 control and 35 preconditioned) where both mean hsTnT and AUC were significantly reduced by RIPC, and therefore it is possible that once again the cohort size might have had an impact on the final outcomes.

In conclusion, it will be essential to confirm whether GTN and NO have a major impact on myocardial and renal injury in the context of cardiac surgery, and whether RIPC-cardio and reno-protection are attenuated with the concomitant GTN administration in a suitably powered prospective RCT trial, for which we strongly believe that our subgroup analysis has given a crucial suggestion.

CHAPTER 6

6. Effects of multi-limb RIPC on cardioprotection in diabetic and non-diabetic patients undergoing cardiac surgery

6.1. Introduction: DM and IHD

DM is the most potent cardiovascular risk factor with devastating multi-system effects (522): 381 million people in the world had DM which also caused 4.6 million deaths in 2011, with a projected prevalence of almost double by 2030 (523), due to a rapidly increasing incidence in the developing world, particularly in Asia and Africa (523, 524): 90% of subjects with DM have Type 2 DM with the remaining 10% being affected by Type 1 DM (523). Macrovascular disease, including CAD, stroke and PVD occurs in 20% of patients with Type 2 DM and is responsible for 59% of death in these subjects (525): in particular, patients with type 2 DM without previous MI have been observed to have the same risk of sustaining an MI than non-diabetic subjects with previous MI (526), with increased rates of subsequent LV failure, re-infarction and death (527-529). Crucially, compared to non-diabetics, diabetic subjects have higher incidence of multi-vessel CAD (527) and worse clinical outcomes following coronary revascularization, with increased rates of in-stent restenosis in subjects undergoing PCI (530, 531) and higher short and long-term mortality in those undergoing CABG (532, 533).

It is therefore clear that DM represents one of the most important challenges of healthcare systems in the UK and the world and that, whilst a crucial component of

these patients' management is an aggressive glycaemic control and prevention of both acute and chronic complications, new protective strategies are required in diabetic patients undergoing revascularisation in order to improve their morbidity and mortality.

6.2. DM, IRI and IPC: where we stand

Animal studies have showed that the diabetic myocardium may have an increased resistance to IRI compared to the non-diabetic heart, although significant differences in results have been obtained from different animal models and with different techniques of diabetes induction (534-543). Whilst this was initially attributed to the acute pharmacological induction of type 1 diabetes in rats, mice, dogs or rabbits through the administration of pancreato-toxic substances including stretozotocin and alloxan (534-543), in contrast with the human model of chronic type 2 diabetes (544), further experimental studies have however confirmed reduced IRI also in animal models of type 2 DM, such as Goto-Kakizaki and Zucker fatty rats (545). The majority of these studies also suggested the possibility of reduced resistance to IRI in animals with a longer history of DM (534, 535). Similarly, IPC has been demonstrated to reduce IRI in animal models of acute type 1 diabetes, however interestingly this effect was lessened in rats which had been diabetic for a few weeks (540). Crucially, in the first animal model of type 2 DM (545), the authors confirmed that chronically diabetic rats sustained less IRI, however they failed to demonstrate reduced myocardial injury by IPC in these models. In a seminal study, our group (440) showed that one or two cycles of IPC were effective in control Wistar rats but not in diabetic Goto-Kakizaki rats, which could only be protected by increasing the stimulus to three cycles. This was therefore the first demonstration that diabetic myocardium requires an increased

preconditioning stimulus and that the threshold required for this stimulus to be protective was higher in diabetic hearts than in non-diabetic hearts. Importantly, the deficient phosphorylation (but not the total amount) of Akt in diabetic rats was identified as the potential reason for the discrepancy between diabetic and non-diabetic myocardium in response to different IPC stimuli intensity, although it could not be clarified whether this could be related to a specific alteration of Akt or whether the potential defect resided upstream. However, subsequently, reduced Akt phosphorylation in diabetic hearts aws found to be secondary to increased levels of phosphatase and tensin homologue on chromosome 10 (PTEN), which negatively regulates PI3-K/Akt pathway (546).

Human studies of the cardioprotective effects of IPC in diabetes have particularly focussed on the analysis of recovery of atrial trabecular contractility in response to an IPC stimulus. Following the concept of an increased preconditioning threshold in the diabetic heart, our group demonstrated that human atrial trabeculae isolated from diabetic patients undergoing CABG surgery recovered a more significant contractile function when subjected to prolonged hypoxic preconditioning (547). In addition, lower levels of phosphorylated Akt were found in diabetic myocardium thereby confirming outcomes of experimental studies. Subsequently, Hassouna and colleagues (548) demonstrated that mitochondrial dysfunction could represent a key pathophysiological factor in diabetic myocardium by proving that mito-K_{ATP} channel opener diazoxide could not protect the diabetic trabeculae, likely due to the lack of mitochondrial membrane depolarisation and ROS production, and that cardioprotection could be re-established with the addition of PKC and p38 activators and superoxide donors, thereby suggesting that these factors are downstream of mito-K_{ATP} channel.

Based on the intriguing results of animal and human studies, we conducted a further retrospective subgroup analysis to evaluate the effects of an enhanced RIPC stimulus on PMI and short-term clinical outcomes in diabetic and non-diabetic patients undergoing elective cardiac surgery: in the first instance we will evaluate results in the context of unselected cardiac surgery to then continue our journey through the different techniques of myocardial preservation and ultimately focus our attention onto CABG surgery with cardioplegia. We did not conduct any further subgroup analysis due to the relatively small sample size. Once again, it is crucial to highlight that our study was not powered for this type of analysis and therefore it will be more appropriate to consider these findings as suggestive of potential effects of RIPC on diabetics.

6.3. Effects of multi-limb RIPC on cardioprotection in diabetic and non-diabetic patients undergoing unselected cardiac surgery

We found 52 subjects with a pre-operative diagnosis of DM, of whom 24 randomised to control and 28 to RIPC **(Table 6.1)**. Of the remaining 126 non-diabetic patients, 65 received the sham protocol and 61 the preconditioning protocol. We found no statistical significant differences in either these subgroups in terms of baseline characteristics or surgical parameters **(Tables 6.1-6.2)**.

Table 6.1. Patient baseline characteristics in diabetic and non-diabetic patients undergoing unselected cardiac surgery

Patients	Diabetics			Non-diabetics		
	Control (n=24)	RIPC (n=28)	P value	Control (n=65)	RIPC (n=61)	P value
Age (*years*)	65±8	65±10	0.981	66±10	64±10	0.208
Gender			0.272			0.826
Male	18 (75.0%)	25 (89.3%)		49 (75.4%)	47 (77.0%)	
Female	6 (25.0%)	3 (10.7%)		16 (24.6%)	14 (23%)	
Ethnicity			0.245			0.443
Caucasian	19 (79.2%)	17 (60.7%)		55 (84.6%)	54 (88.5%)	
Asian	3 (12.5%)	9 (32.1%)		7 (10.8%)	3 (4.9%)	
Afro-Caribbean	1 (4.2%)	2 (7.1%)		3 (4.6%)	4 (6.6%)	
Chinese	1 (4.2%)	0 (0%)		0 (0%)	0 (0%)	
BMI	29.6±6.6	29.6±5.4	0.986	28.0±4.9	28.4±7.8	0.735
SBP *(mmHg)*	133.2±20	128.8±14.7	0.371	128.8±17.5	129.1±16.3	0.937
DBP *(mmHg)*	72.9±10.3	69.7±7.5	0.205	69.9±8.5	71.3±	0.417
HR *(bpm)*	73.5±11.9	66.2±9.0	0.0.17	67.7±11.3	66.3±10.2	0.477
Smoking History			0.247			0.626
Smoker	4 (16.7%)	1 (3.6%)		8 (12.3%)	10 (16.4%)	
Ex-smoker	14 (58.3%)	17 (60.7%)		38 (58.5%)	31 (50.8%)	
Non-smoker	6 (16.7%)	10 (35.7%)		25 (29.3%)	30 (32.8%)	
Family History of IHD	16 (66.7%)	23 (82.1%)	0.220	41 (63.1%)	41 (67.2%)	
						0.335
NYHA Class	2.86±0.8	2.63±0.7	0.287	2.75±0.9	2.48±0.8	0.099
CCS Class	2.45±1.2	2.70±1.1	0.458	2.13±1.1	2.17±1.0	0.807
LVEF			0.954			0.192
>50%	17 (70.8%)	19 (67.9%)		53 (81.5%)	48 (78.7%)	
30%-50%	5 (20.8%)	6 (21.4%)		12 (18.5%)	10 (16.4%)	
<30%	2 (8.3%)	3 (10.7%)		0 (0.0%)	3 (4.9%)	
Co-morbidities						
Hypertension	19 (79.2%)	25 (89.3%)	0.447	51 (78.5%)	40 (65.6%)	0.116
Hypercholesterolemia	19 (79.2%)	25 (89.3%)	0.608	45 (69.2%)	43 (70.5%)	0.878
Atrial Fibrillation	3 (12.5%)	2 (7.1%)	0.652	13 (20.0%)	8 (13.1%)	0.497
Previous MI	4 (16.7%)	1 (39.3%)	0.124	19 (29.2%)	17 (27.9%)	0.866
Previous PCI	5 (20.8%)	6 (21.4%)	1.000	6 (9.2%)	5 (8.2%)	0.837
Previous CVA/TIA	2 (8.4%)	1 (3.6%)	0.546	7 (10.8%)	4 (6.6%)	0.062
Previous Cardiac Surgery	1 (4.2%)	2 (7.1%)	1.000	1 (1.5%)	2 (3.2%)	0.584
Other comorbidities	8 (34.7%)	11 (39.3%)	0.920	6 (9.2%)	2 (3.3%)	0.384
Peripheral Arterial Disease	2 (8.3%)	1 (3.6%)	0.590	4 (6.2%)	0 (0.0%)	0.120
Drug History	17 (77.3%)	24 (85.7%)	0.489	49 (75.4%)	48 (83.8%)	0.606
Aspirin	6 (27.3%)	10 (35.7%)	0.559	21 (32.3%)	14 (24.1%)	0.158
Clopidogrel/Prasugrel	2 (9.1%)	2 (7.1%)	0.127	7 (10.8%)	4 (6.9%)	0.308
Warfarin	15 (68.1%)	21 (75%)	0.426	40 (61.5%)	36 (62.1%)	0.952
Beta-blocker	10 (45.5%)	7 (25.0%)	0.098	22 (33.8%)	15 (25.9%)	0.448
Calcium Channel Blocker	19 (86.1%	25 (89.2%)	0.096	53 (81.5%)	47 (81.0%)	0.211
Statin	17 (74.9%)	21 (75%)	0.797	44 (67.7%)	35 (60.3%)	0.383
ACE-I/ARB	8 (36.3%)	5 (17.9%)	0.241	6 (9.2%)	7 (12.1%)	0.467
Long acting nitrates						
Antidiabetics						
Insulin	7 (31.8%)	8 (28.6%)	0.522			
Biguanide	16 (72.7%)	16 (57.1%)	0.522			
Sulphonylurea	11 (50%)	6 (21.4%)	0.060			
Diuretics	7 (31.8%)	13 (46.4%)	0.512	20 (30.8%)	18 (31.0%)	0.600

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA=Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF=left ventricular ejection fraction.

Table 6.2. Details of surgical procedure in diabetic and non-diabetic patients undergoing unselected cardiac surgery

Patients	Diabetics			Non-diabetics		
	Control (n=24)	RIPC (n=28)	P value	Control (n=65)	RIPC (n=61)	P value
Indication for Surgery			0.174			0.817
Angina	17 (70.8%)	14 (50.0%)		27 (41.5%)	26 (42.6%)	
Myocardial Infarction	0 (0.0%)	6 (21.4%)		12 (18.5%)	13 (21.3%)	
Valve Disease	4 (16.7%)	23 (25.8%)		19 (29.2%)	19 (31.1%)	
Angina and Valve Disease	7 (7.9%)	4 (14.3%)		5 (7.7%)	2 (3.3%)	
MI and Valve Disease	2 (8.3%)	2 (7.1%)		0 (0.0%)	0 (0.0%)	
Infective Endocarditis	2 (2.2%)	1 (1.1%)		2 (3.1%)	1 (1.6%)	
EuroSCORE	3.21±1.87	4.00±3.31	0.304	3.91±2.07	3.56±2.2	0.359
Additive perioperative risk			0.356			0.356
Low (EuroSCORE 0-2)	26 (29.2%)	29 (32.6%)		26 (29.2%)	29 (32.6%)	
Medium (EuroSCORE 3-5)	47 (52.8%)	38 (42.7%)		47 (52.8%)	38 (42.7%)	
High (EuroSCORE >5)	16 (18%)	22 (24.7%)		16 (18%)	22 (24.7%)	
Bypass-time (min)	101.50±27.75	91.85±29.12	0.245	95.1±34.2	88.7±32.0	0.360
Cross-clamp time (min)	63.4±23.1	65.0±25.5	0.826	65.2±25.6	59.9±25.6	0.286
Cardioprotection						
Blood cardioplegia	18 (75.0%)	23 (82.1%)	0.073	55 (84.6%)	52 (85.2%)	0.828
Cross-clamp fibrillation	6 (25.0%)	5 (17.9%)		16 (18.0%)	14 (15.9%)	0.842
Operation			0.922			0.845
CABG alone	17 (70.8%)	19 (67.9%)		37 (56.9%)	38 (62.3%)	
AVR alone	2 (8.3%)	2 (7.1%)		13 (20.0%)	12 (19.7%)	
CABG+AVR	4 (16.7%)	5 (17.9%)		6 (9.2%)	4 (6.6%)	
MVR or MV Repair	1 (4.2%)	1 (3.6%)		8 (12.3%)	7 (11.5%)	
AVR+MVR	0 (0.0%)	1 (3.6%)		1 (1.5%)	0 (0.0%)	
Number of grafts			0.427			0.982
One	1 (4.2%)	3 (10.7%)		3 (4.6%)	2 (3.3%)	
Two	8 (33.3%)	5 (17.9%)		11 (16.9%)	10 (16.4%)	
Three	7 (29.2%)	13 (46.4%)		22 (33.8%)	22 (36.1%)	
Four	5 (20.8%)	3 (10.7%)		7 (10.8%)	8 (13.1%)	
Anesthetic agents						
Induction			0.992			0.152
Anti-nicotinic agents						
Rocuronium	18 (81.8%)	23 (85.2%)		50 (80.6%)	53 (91.4%)	
Pancuronium	3 (13.6%)	3 (11.1%)		11 (17.7%)	3 (5.2%)	
Vecuronium	2 (2.4%)	3 (3.6%)		1 (1.6%)	2 (3.4%)	
Midazolam	9 (40.9%)	12 (44.4%)	0.804	36 (58.1%)	21 (36.2%)	0.019
Etomidate	0 (0.0%)	3 (11.1%)	0.805	8 (12.5%)	4 (6.9%)	0.299
Fentanyl	24 (100%)	28 (100%)	1.000	65 (100%)	61 (100%)	1.000
Propofol	20 (90.9%)	24 (88.9%)	0.816	56 (87.5%)	53 (91.4%)	0.566
Maintenance						
Propofol	24 (100%)	28 (100%)	1.000	65 (100%)	61 (100%)	1.000
Volatile Anesthetics			0.830			0.527
Isoflurane	20 (90.9%)	25 (92.6%)		60 (93.8%)	56 (96.6%)	
Sevoflurane	2 (9.1%)	2 (7.4%)		4 (6.3%)	2 (3.4%)	
Intra-operative GTN	19 (79.2%)	18 (64.3%)	0.238	46 (74.2%)	35 (58.3%)	0.840

RIPC= Remote Ischemic Preconditioning; CABG= Coronary artery bypass graft; AVR=Aortic valve replacement; MVR= Mitral valve replacement; MV= Mitral valve; MI=myocardial infarction.

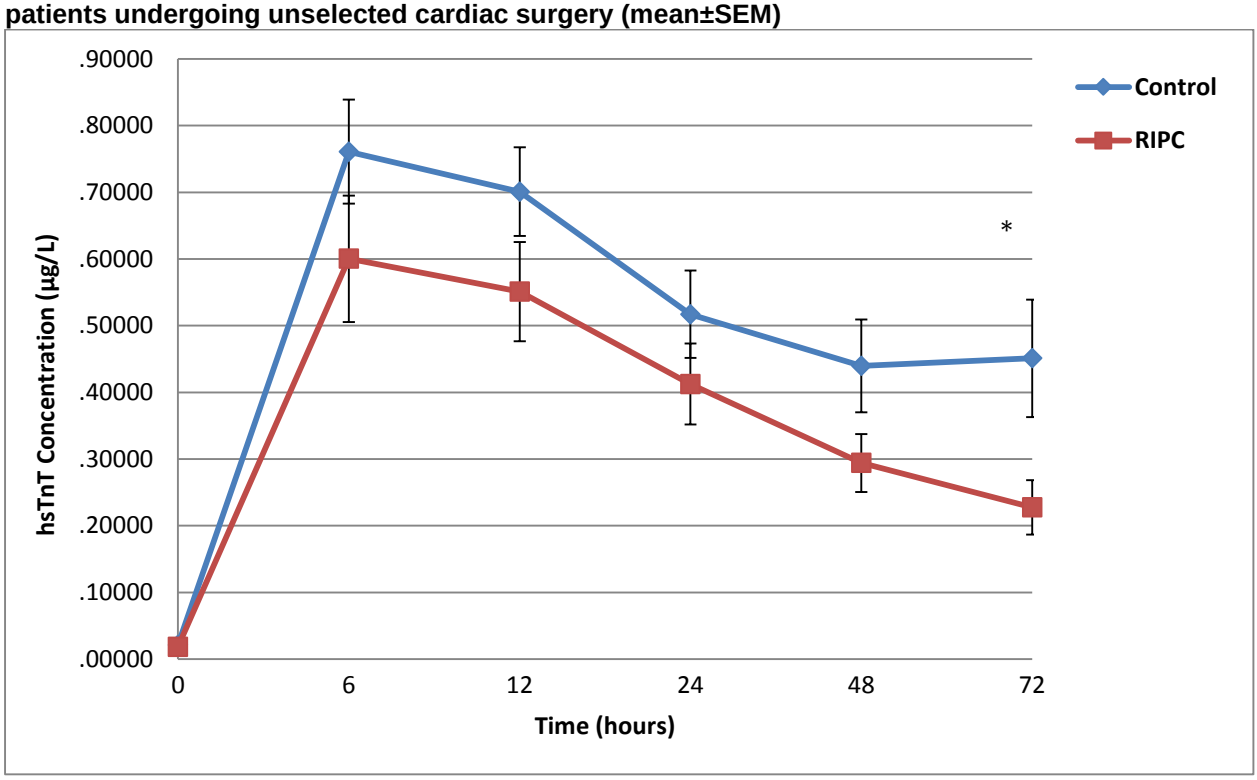
In the diabetic groups, preconditioned patients had lower mean hsTnT levels at all the specified time points, however this reached statistically significance only at 72 hours post-operatively **(Fig. 6.1, Table 6.3)**. Similarly RIPC reduced total AUC from 35.993±21.859 µg/L to 25.927±20.031 µg/L, which failed to reach statistical significance [10.07; CI -1.844; 21.00; p=0.096] **(Fig. 6.2, Table 6.3)**.

Table 6.3. High-sensitivity Troponin-T release pre-operatively and at 6, 12, 24, 48 and 72 hours post-operatively in diabetic and non-diabetic patients undergoing unselected cardiac surgery

Endpoint		Control DM: n=24 Non-DM: n=65 (mean (sd))	RIPC DM: n=28 Non-DM: n=61 (mean (sd))	Difference (95% CI)	P value
hsTnT (µg/L)					
Pre-operatively	DM	0.021 (0.023)	0.018 (0.025)	0.003 (0.007, -0.011)	0.682
	No-DM	0.017 (0.017)	0.013 (0.018)	0.003 (-0.003, -0.009)	0.275
6 hours post-operatively	DM	0.761 (0.383)	0.600 (0.502)	0.161 (-.0.091, 0.413)	0.206
	No-DM	0.817 (0.536)	0.620 (0.316)	0.197 (0.043, 0.352)	0.013
12 hours post-operatively	DM	0.701 (0.325)	0.551 (0.394)	0.149 (0.101, -0.053)	0.145
	No-DM	0.712 (0.475)	0.558 (0.371)	0.154 (0.004, 0.304)	0.044
24 hours post-operatively	DM	0.517 (0.321)	0.413 (0.322)	0.104 (-0.075, 0.283)	0.248
	No-DM	0.535 (0.351)	0.406 (0.242)	0.129 (0.022, 0.135)	0.018
48 hours post-operatively	DM	0.440 (0.342)	0.294 (0.225)	0.146 (-0.015, 0.307)	0.075
	No-DM	0.440 (0.432)	0.313 (0.193)	0.127 (0.007- 0.248)	0.035
72 hours post-operatively	DM	0.451 (0.432)	0.227 (0209)	0.224 (0.033, 0.414)	0.022
	No-DM	0.389 (0.314)	0.299 (0.223)	0.091 (-0.007, 0.188)	0.068
Total 72 hours AUC	DM	35.993 (21.859)	25.927 (20.031)	10.07 (-1.844, 21.00)	0.096
	No-DM	36.428 (25.673)	27.479 (14.899)	8.949 (1.423, 16.477)	0.020

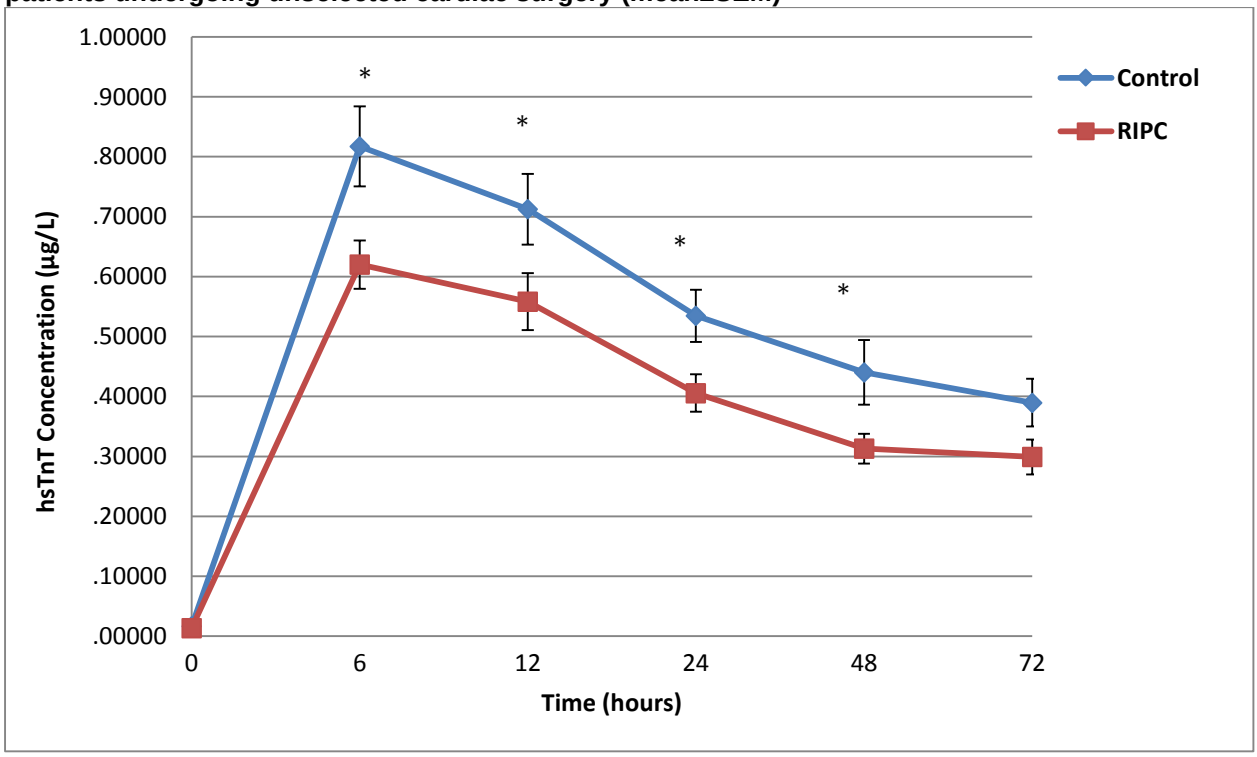
RIPC=Remote ischemic preconditioning; SD=standard deviation; CI=confidence interval; hsTnT=high sensitivity Troponin-T

Fig. 6.1. Mean high-sensitivity Troponin-T levels at 0, 6, 12, 24, 48 and 72 hours in diabetic patients undergoing unselected cardiac surgery (mean±SEM)



RIPC=remote ischaemic preconditioning; hsTnT=high sensitivity troponin-T; SEM=standard error of the mean. *p<0.05 (unpaired Student T-Test)

Fig. 6.2. Mean high-sensitivity Troponin-T levels at 0, 6, 12, 24, 48 and 72 hours in non-diabetic patients undergoing unselected cardiac surgery (mean±SEM)

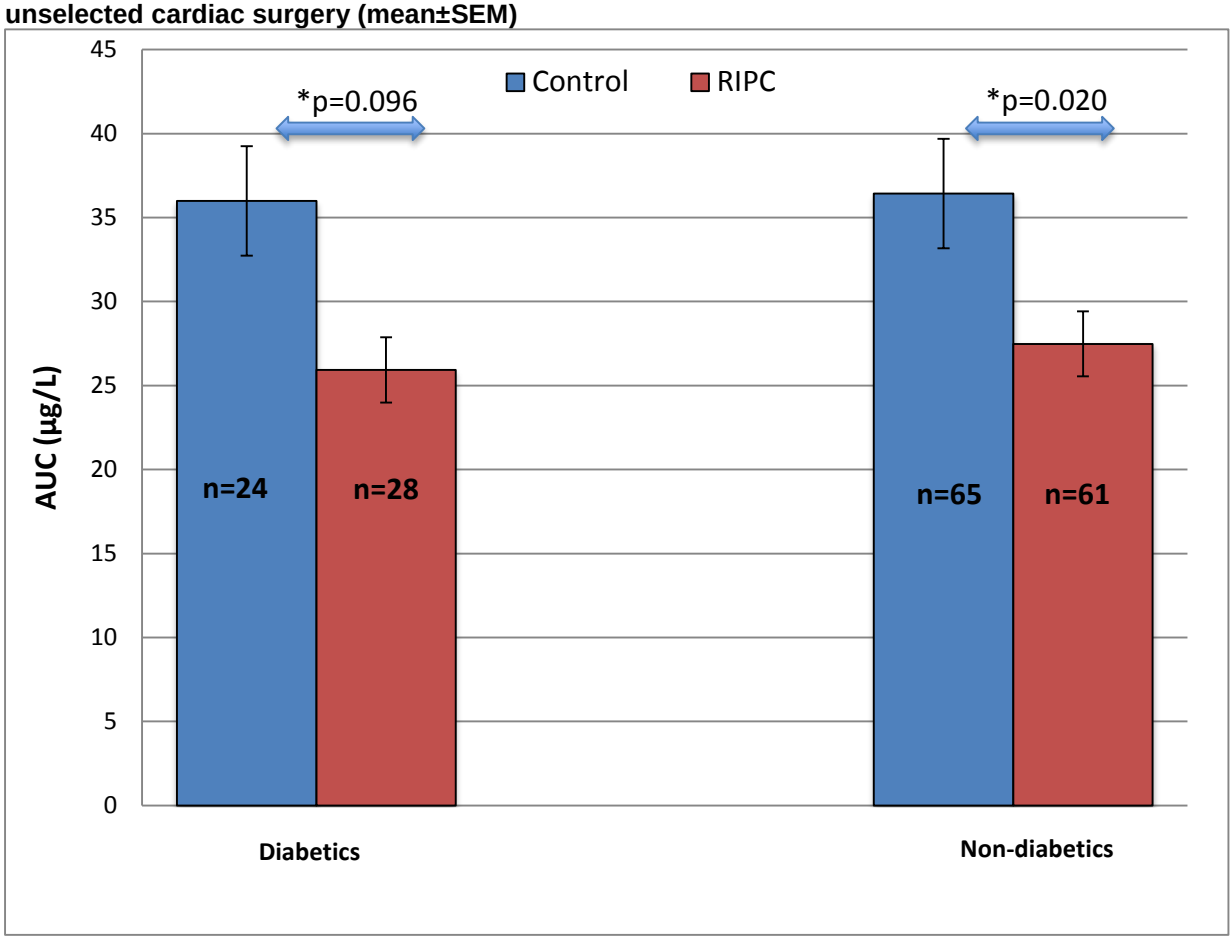


RIPC=remote ischaemic preconditioning; hsTnT=high sensitivity troponin-T; SEM=standard error of the mean. *p<0.05 (unpaired Student T-Test)

Conversely and similarly to our general cohort, RIPC significantly reduced mean hsTnT in non-diabetic patients at all the different time points and lessened total AUC from 36.428±25.673 µg/L to 27.479±14.899µg/L, which corresponded to a significant 25% reduction [8.949; CI 1.423, 16.477; p=0.020] **(Fig. 6.3, Table 6.3)**.

We found no difference in secondary endpoints between control and preconditioned patients in either diabetic or diabetic subgroups **(Tables 6.4-6.5)**.

Fig. 6.3. Total AUC in control and RIPC diabetic and non-diabetic patients undergoing unselected cardiac surgery (mean±SEM)



RIPC=remote ischaemic preconditioning; SEM=standard error of the mean; AUC=area-under-the-curve
*Unpaired Student T-Test

Table 6.4. Summary of major secondary endpoints in diabetic patients undergoing unselected cardiac surgery*

Endpoint	Control (n=24) (mean (sd))	RIPC (n=28) (mean (sd))	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	30650.65 (17259.69)	35084.74 (25290.42)	-4434.09 (-18850, 9982.36)	0.537
Creatinine (mg/ml)				
Pre-operatively	92.67 (21.51)	96.14 (36.75)	-3.476 (-20.627, 13.674)	0.686
24 hours post-operatively	97.79 (37.08)	96.48 (33.82)	1.310 (-18.635, 21.255)	0.896
48 hours post-operatively	112.54 (47.45)	108.36 (53.28)	4.185 (-24.132, 32.501)	0.768
72 hours post-operatively	112.25 (62.25)	106.93 (62.55)	5.321 (-29.55, 40.193)	0.760
Urine Output (ml)				
24 hours post-operatively	1874.6 (667.2)	2171.4 (626.7)	-113.04 (-521.84, 295.755)	0.580
48 hours post-operatively	2036.1 (876.9)	2351.8 (903.1)	-315.65 (-872.70, 241.400)	0.257
72 hours post-operatively	1935.9 (1071.9)	2715.8 (883.4)	-779.89 (-1524.3, -35.44)	0.041
Total	5571.9 (1650.6)	6722.4 (1413.2)	-1150.5 (-2317.6, 16.517)	0.058
AKI score				
0	71	80		0.12
1	8	5		
2	4	1		
3	3	0		
Acute Kidney Injury (total)	2	6		0.208
Inotrope score				
Post bypass	5.61 (12.57)	4.45 (9.63)	1.16 (-5.279, 7.592)	0.719
24 hours post-operatively	9.04 (18.90)	8.38 (13.69)	0.661 (-8.808, 10.131)	0.893
48 hours post-operatively	10.52 (22.53)	4.80 (12.87)	5.722 (-4.661, 16.105)	0.273
72 hours post-operatively	8.55 (20.99)	0.57 (2.45)	7.981 (-0.196, 16.157)	0.098
Total	33.83 (12.56)	18.08 (5.99)	15.75 (-10.37, 41.87)	0.231
New onset AF	7	3		0.157
Length of ICU stay (days)	3.0 (2.0-5.5)**	2.0 (1.0-3.5)**		0.567***
Length of hospital stay (days)	9.0 (7.5-11.5)**	8.0 (6.0-10.5)**		0.784***
Clinical outcomes at six weeks				
Death	1	0		0.462
Myocardial infarction	0	0		1.000
Stroke	0	0		1.000
Revascularization	0	0		1.000

Table 6.5. Summary of major secondary endpoints in non-diabetic patients undergoing unselected cardiac surgery*

Endpoint	Control (n=65) (mean (sd))	RIPC (n=61) (mean (sd))	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	33290.79 (20455.04)	36984.57 (28928.43)	-3693.78 (-14327.34, 6939.78)	0.492
Creatinine (mg/ml)				
Pre-operatively	84.9 (18.7)	81.4 (20.3)	3.56 (3.31, 10.43)	0.307
24 hours post-operatively	90.35 (27.1)	83.8 (22.7)	6.62 (-2.21, 15.44)	0.140
48 hours post-operatively	99.9 (49.9)	84.6 (26.8)	15.23 (1.18, 29.26)	0.034
72 hours post-operatively	93.9 (48.2)	84.2 (27.9)	9.73 (-4.28, 23.74)	0.172
Urine Output (ml)				
24 hours post-operatively	2030.1 (797.7)	2255.2 (571.6)	-225.13 (-481.13, 30.88)	0.084
48 hours post-operatively	2181.4 (941.3)	2348.7 (829.9)	-167.26 (-515.57, 181.06)	0.343
72 hours post-operatively	2057.7 (785.9)	2386.7 (801.8)	-329.00 (-696.93, 38.93)	0.079
Total	5958.7 (1792.8)	6700.0 (1665.7)	-741.35 (-1543.36, 60.66)	0.069
AKI score				
0	71	80		0.12
1	8	5		
2	4	1		
3	3	0		
Acute Kidney Injury (total)	3	0		0.236
Inotrope score				
Post bypass	7.2 (13.9)	7.9 (17.2)	-0.69 (-6.32, 4.92)	0.806
24 hours post-operatively	12.5 (21.7)	9.9 (17.8)	2.59 (-4.60, 9.79)	0.477
48 hours post-operatively	7.7 (17.9)	5.9 (14.8)	1.88 (-4.11, 7.86)	0.536
72 hours post-operatively	4.6 (15.2)	2.2 (9.9)	2.41 (-2.27, 7.09)	0.311
Total	32.30 (59.69)	24.80 (46.68)	7.50 (-12.02, 27.02)	0.448
New onset AF	15	7		0.086
Length of ICU stay (days)	3.0 (2.0-4.0)**	2.0 (1.0–4.0)**		0.313***
Length of hospital stay (days)	8.0 (7.0–12.0)**	8.0 (6.0–10.0)**		0.078***
Clinical outcomes at six weeks				
Death	1	0		0.331
Myocardial infarction	1	0		0.331
Stroke	0	2		0.214
Revascularization	0	0		1.000

*List of abbreviations.
RIPC=Remote ischemic preconditioning; CK=Creatinine Kinase; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve. **Results shown as median (inter-quartile range); *** P-value for Mann-Whitney-Wilcoxon test

6.4. Effect of multi-limb RIPC on cardioprotection in diabetic and non-diabetic patients undergoing unselected cardiac surgery with cardioplegia

In our main cohort analysis we found that RIPC reduced total hsTnT AUC irrespective of the technique of myocardial preservation. Hence we intended to establish whether the protective effects of our enhanced preconditioning stimulus would be beneficial to diabetic and/or non-diabetic subjects undergoing unselected cardiac surgery with cardioplegia. We did not perform subgroup analysis of ICCF patients with or without DM due their small sample size.

Diabetic patients were 18 and 23 in control and RIPC groups respectively and non-diabetic subjects were 23 and 52 respectively **(Table 6.6)**. Preconditioned patients had a lower mean heart rate within the diabetic subgroup (64.8±8.5 versus 71.9±12.1; p=0.033) and a less significant pre-operative history of CVA within the non-diabetic subgroup (3 versus 6 cases; p=0.038) **(Table 6.6)**. We found no other significant difference between control and RIPC patients in any of the subgroups with regards to other baseline or surgical parameters **(Tables 6.6-6.7)**.

Table 6.6. Baseline characteristics in diabetic and non-diabetic patients undergoing cardiac surgery with cardioplegia

Patients	Diabetics			Non-diabetics		
	Control (n=18)	RIPC (n=23)	P value	Control (n=55)	RIPC (n=52)	P value
Age (*years*)	65±7	67±10	0.517	68±11	65±10	0.160
Gender			0.438			0.863
Male	14 (77.8%)	20 (87.0%)		41 (74.5%)	38 (73.1%)	
Female	4 (22.2%)	3 (13.0%)		14 (25.5%)	14 (26.9%)	
Ethnicity			0.219			0.443
Caucasian	14 (77.8%)	13 (56.5%)		47 (85.5%)	45 (86.5%)	
Asian	2 (11.1%)	8 (34.8%)		6 (10.9%)	3 (5.8%)	
Afro-Caribbean	1 (5.6%)	2 (8.7%)		2 (3.6%)	4 (7.7%)	
Chinese	1 (5.6%)	0 (0%)		0 (0%)	0 (0%)	
BMI	29.9±6.6	29.1±5.4	0.687	27.4±5.0	28.5±8.4	0.433
SBP *(mmHg)*	133.6±19.4	129.5±14.8	0.447	129.2±18.1	130.0±17.5	0.918
DBP *(mmHg)*	71.7±8.8	70.7±7.5	0.675	69.7±8.7	71.3±10.7	0.420
HR *(bpm)*	71.9±12.1	64.8±8.5	0.033	68.4±11.9	67.1±10.2	0.545
Smoking History			0.105			0.452
Smoker	3 (16.7%)	0 (0%)		5(9.1%)	9 (17.3%)	
Ex-smoker	10 (55.6%)	13 (56.5%)		34 (61.8%)	29 (55.8%)	
Non-smoker	5 (27.8%)	10 (43.5%)		16 (29.1%)	14 (26.9%)	
Family History of IHD	13 (72.2%)	18 (78.3%)	0.655	31 (56.4%)	36 (69.2%)	0.169
NYHA Class	3.00±0.69	2.73±0.70	0.225	2.83±0.95	2.52±0.79	0.071
CCS Class	2.44±1.2	2.68±1.1	0.532	2.07±1.1	2.06±1.0	0.948
LVEF			0.721			0.326
>50%	13 (72.2%)	15 (65.2%)		45 (81.8%)	42 (80.8%)	
30%-50%	4 (22.2%)	5 (21.7%)		10 (18.2%)	8 (15.4%)	
<30%	1 (5.6%)	3 (13.0%)		0 (0%)	2 (3.8%)	
Co-morbidities						
Hypertension	14 (77.8%)	20 (87.0%)	0.438	43 (78.2%)	36 (69.2%)	0.292
Hypercholesterolemia	14 (77.8%)	20 (87.0%)	0.438	35 (63.6%)	36 (69.2%)	0.540
Atrial Fibrillation	3 (16.7%)	1 (4.3%)	0.187	13 (23.6%)	8 (15.4%)	0.484
Previous MI	4 (22.2%)	9 (39.1%)	0.248	16 (29.1%)	24 (26.9%)	0.803
Previous PCI	4 (22.2%)	5 (21.7%)	0.970	6 (10.9%)	3 (5.8%)	0.038
Previous CVA/TIA	2 (11.2%)	1 (4.3%)	0.507	7 (12.4%)	4 (7.7%)	0.061
Previous Cardiac Surgery	1 (5.6%)	2 (8.7%)	0.702	1 (1.8%)	2 (3.8%)	0.586
Other comorbidities	1 (5.6%)	2 (3.9%)	0.837	5 (9.1%)	2 (3.9%)	0.562
Peripheral Arterial Disease	2 (11.2%)	1 (4.3%)	0.905	3 (5.5%)	0 (0.0%)	0.088
Drug History						
Aspirin	14 (77.8%)	19 (82.6%)	0.923	40 (72.7%)	40 (80.0%)	0.683
Clopidogrel/Prasugrel	6 (33.3%)	8 (34.8%)	0.184	18 (32.7%)	10 (20.0%)	0.160
Warfarin	2 (11.1%)	1 (4.3%)	0.493	7 (12.7%)	4 (8.0%)	0.300
Beta-blocker	12 (66.7%)	16 (69.6%)	0.136	32 (58.2%)	29 (58.0%)	0.985
Calcium Channel Blocker	7 (38.9%)	6 (26.1%)	0.580	20 (36.3%)	14 (28.0%)	0.464
Statin	16 (81.4%)	20 (69.5%)	0.191	43 (78.2%)	40 (80.0%)	0.191
ACE-I/ARB	15 (65.5%)	31 (62.0%)	0.332	36 (65.5%)	31 (62.0%)	0.382
Long acting nitrates	7 (38.9%)	5 (21.7%)	0.566	5 (9.1%)	5 (10.0%)	0.566
Antidiabetics						
Insulin	4 (22.2%)	5 (21.7%)	0.515			
Biguanide	7 (38.9%)	12 (52.1%)	0.689			
Sulphonylurea	9 (50.0%)	6 (26.1%)	0.136			
Diuretics	7 (38.9%)	10 (43.5%)	0.418	16 (29.1%)	16 (32.0%)	0.805

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA=Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF=left ventricular ejection fraction.

Table 6.7. Details of surgical procedure in diabetic and non-diabetic patients undergoing cardiac surgery with cardioplegia

Patients	Diabetics			Non-diabetics		
	Control (n=18)	RIPC (n=23)	P value	Control (n=55)	RIPC (n=52)	P value
Indication for Surgery						
Angina			0.198			0.779
Myocardial Infarction	11 (61.1%)	9 (39.1%)		20 (36.4%)	19 (36.5%)	
Valve Disease	0 (0.0%)	6 (26.1%)		9 (16.4%)	11 (21.2%)	
Angina and Valve Disease	4 (22.2%)	4 (17.4%)		19 (34.5%)	19 (36.5%)	
MI and Valve Disease	2 (11.1%)	2 (8.7%)		5 (9.1%)	2 (3.8%)	
Infective Endocarditis	1 (5.6%)	2 (8.7%)		2 (3.6%)	1 (1.9%)	
EuroSCORE	3.33±1.9	4.70±3.2	0.121	4.15±2.04	3.73±2.14	0.308
Additive perioperative risk			0.120			0.545
Low (EuroSCORE 0-2)	7 (38.9%)	5 (21.7%)		11 (20.0%)	15 (28.8%)	
Medium (EuroSCORE 3-5)	9 (50.0%)	9 (39.1%)		31 (56.4%)	25 (48.11%)	
High (EuroSCORE >5)	2 (11.1%)	9 (39.1%)		13 (23.6%)	12 (23.1%)	
Bypass-time (min)	102.44±28.71	96.55±30.07	0.533	99.78±34.66	90.06±33.89	0.146
Cross-clamp time (min)	67.67±21.97	72.23±22.55	0.524	71.52±25.33	64.06±27.73	0.151
Number of grafts			0.505			0.923
One	3 (16.7%)	4 (17.4%)		22 (40.0%)	19 (36.5%)	
Two	1 (5.6%)	3 (13.0%)		3 (5.5%)	2 (3.8%)	
Three	7 (38.9%)	4 (17.4%)		9 (16.4%)	10 (19.2%)	
Four	4 (22.2%)	9 (39.1%)		15 (27.3%	17 (32.7%)	
	3 (16.7%)	3 (13.0%)		6 (10.9%)	4 (7.7%)	
Operation			0.928			0.827
CABG alone	11 (61.1%)	14 (60.9%)		27 (49.1%)	29 (55.8%)	
AVR alone	2 (11.1%)	2 (8.7%)		13 (23.6%)	12 (23.1%)	
CABG+AVR	4 (22.2%)	5 (21.7%)		6 (10.9%)	4 (7.7%)	
MVR or MV Repair	1(5.6%)	1 (4.3%)		7 (77.8%)	7 (87.5%)	
AVR+MVR	0 (0.0%)	1 (4.3%)		2 (22.2%)	1 (12.5%)	
Anesthetic agents						
Induction						
Anti-nicotinic agents			0.712			0.224
Rocuronium	14 (77.8%)	19 (86.4%)		42 (79.2%)	46 (92.0%)	
Pancuronium	3 (16.7%)	2 (9.1%)		10 (18.9%)	3 (6.0%)	
Vecuronium	1 (5.6%)	1 (4.5%)		1 (1.9%)	1 (2.0%)	0.089
Midazolam	9 (50.0%)	9 (40.9%)	0.565	29 (54.7%)	19 (38.0%)	0.591
Etomidate	0 (0.0%)	3 (13.6%)	0.103	6 (11.1%)	4 (8.0%)	1.000
Fentanyl	18 (100%)	23 (100%)	1.000	54 (100%)	50 (100%)	0.854
Propofol	16 (88.9%)	19 (86.4%)	0.810	48 (88.9%)	45 (90.0%)	
Maintenance						
Propofol	18 (100%)	23 (100%)	1.00	54 (100%)	50 (100%)	1.00
Volatile Anesthetics						
Isoflurane	16 (88.9%)	21 (95.5%)	0.433	51 (94.4%)	49 (98.0%)	0.274
Sevoflurane	3 (5.6%)	1 (2.0%)	0.346	3 (5.6%)	1 (2.0%)	0.346
Intra-operative GTN	14 (77.8%)	13 (56.5%)	0.154	37 (71.2%)	28 (54.9%)	0.087

RIPC=Remote Ischemic Preconditioning; CABG=Coronary artery bypass graft; AVR=Aortic valve replacement; MVR=Mitral valve replacement; MV=Mitral valve; MI=myocardial infarction.

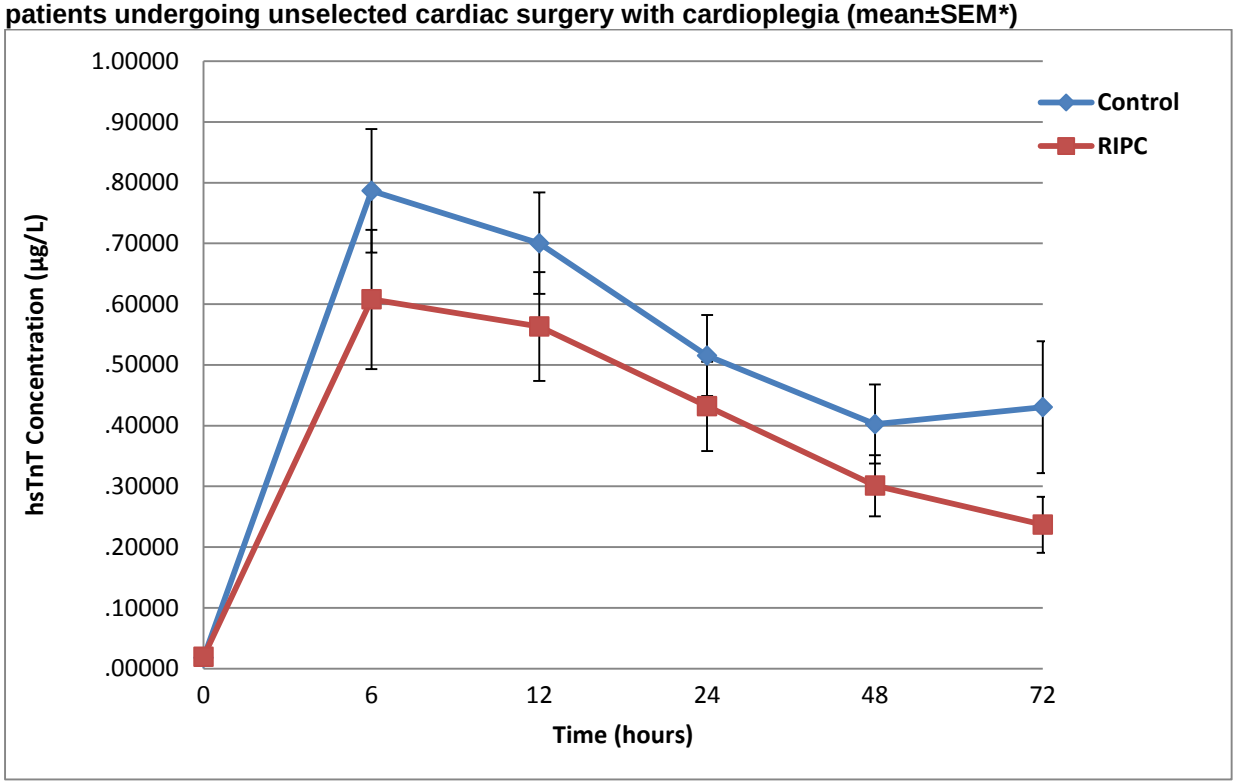
Diabetic preconditioned patients had lower mean hsTnT concentrations at all the time points without however reaching statistical significance **(Fig. 6.4, Table 6.8)**, in contrast with the significantly lower hsTnT at 6, 24 hours post-operatively in the non-diabetic group **(Fig. 6.5, Table 6.8)**. RIPC reduced total AUC in diabetics from 34.88±20.576 μg/L to 26.59±21.23 μg/L [7.51; CI 0.066, 21.029; p=0.216] and in non-diabetics from 37.85±27.43 μg/L to 28.55±15.55 μg/L [9.30; CI 0.58, 18.02; p=0.037], which corresponded to a non-significant reduction of 24% in the former and a significant decrease of 25% in the latter **(Fig. 6.6, Table 6.8)**.

Table 6.8. Mean high-sensitivity Troponin-T pre-operatively and at 6, 12, 24, 48 and 72 hours post-operatively in diabetic and non-diabetic patients undergoing unselected cardiac surgery with cardioplegia

Endpoint		Control DM: n=18 Non-DM: n=55 (mean (sd))	RIPC DM: n=23 Non-DM: n=52 (mean (sd))	Difference (95% CI)	P value
hsTnT *(μg/L)*					
Pre-operatively	DM	0.018 (0.019)	0.019 (0.026)	-0.002 -0.017, 0.013)	0.825
	No-DM	0.017 (0.018)	0.014 (0.019)	0.035 (-0.004, 0.010)	0.417
6 hours post-operatively	DM	0.786 (0.432)	0.608 (0.550)	0.1788 (-0.141, 0.498)	0.265
	No-DM	0.838 (0.571)	0.644 (0.326)	0.194 (-0.017, 0.371)	0.032
12 hours post-operatively	DM	0.700 (0.354)	0.563 (0.429)	0.137 (-0.116, 0.391)	0.280
	No-DM	0.735 (0.509)	0.580 (0.390)	0.156 (-0.018, 0.329)	0.078
24 hours post-operatively	DM	0.515 (0.283)	0.432 (0.353)	0.084 (-0.123, 0.290)	0.417
	No-DM	0.550 (0.371)	0.424 (0.253)	0.126 (0.004, 0.248)	0.042
48 hours post-operatively	DM	0.403 (0.276)	0.301 (0.241)	0.102 (-0.062, 0.265)	0.217
	No-DM	0.462 (0.463)	0.330 (0.203)	0.132 (-0.006, 0.269)	0.061
72 hours post-operatively	DM	0.431 (0.460)	0.237 (0.221)	0.187 (-0.017, 0.411)	0.083
	No-DM	0.407 (0.332)	0.314 (0.234)	0.923 (-0.020, 0.205)	0.107
Total 72 hours AUC	DM	34.88 (20.576)	26.59 (21.23)	7.51 (0.066, 21.029)	0.216
	No-DM	37.85 (27.43)	28.55 (15.55)	9.30 (0.58, 18.02)	0.037

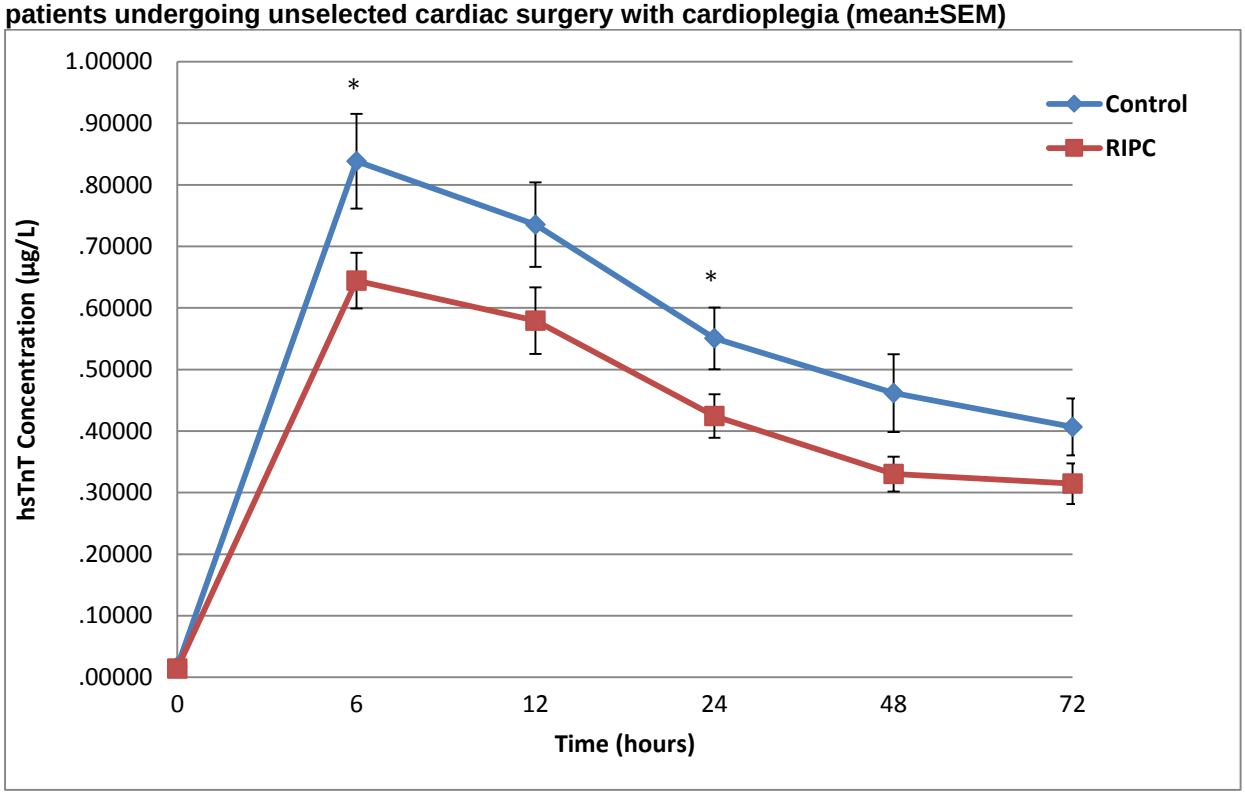
RIPC=Remote ischemic preconditioning; SD= standard deviation; CI= confidence interval; hsTnT= high sensitivity Troponin-T

Fig. 6.4. Mean high-sensitivity Troponin-T levels at 0, 6, 12, 24, 48 and 72 hours in diabetic patients undergoing unselected cardiac surgery with cardioplegia (mean±SEM*)



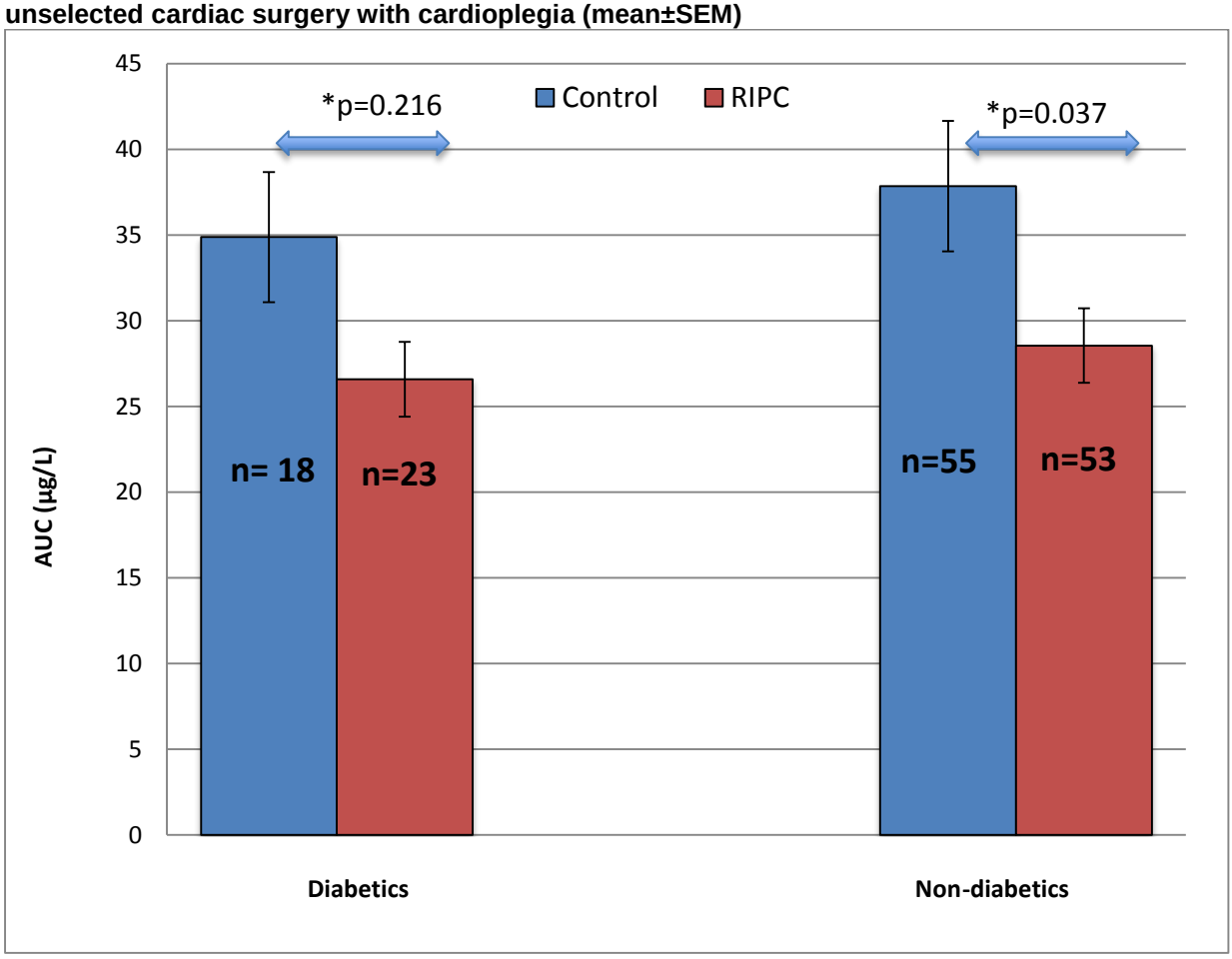
RIPC=remote ischaemic preconditioning; hsTnT=high sensitivity troponin-T; SEM=standard error of the mean. *Unpaired Student T-Test

Fig. 6.5. Mean high-sensitivity Troponin-T levels at 0, 6, 12, 24, 48 and 72 hours in non-diabetic patients undergoing unselected cardiac surgery with cardioplegia (mean±SEM)



RIPC=remote ischaemic preconditioning; hsTnT=high sensitivity troponin-T; SEM=standard error of the mean. *p<0.05 (unpaired Student T-Test)

Fig. 6.6. Total AUC in control and RIPC diabetic and non-diabetic patients undergoing unselected cardiac surgery with cardioplegia (mean±SEM)



RIPC=remote ischaemic preconditioning; SEM=standard error of the mean; AUC=area under the curve
* Unpaired Student T-Test

Similarly we found no statistically significant difference in any of the secondary endpoints between control and preconditioned patients in either the diabetic or non-diabetic subgroups **(Tables 6.9-6.10)**.

Table 6.9. Summary of major secondary endpoints in diabetic patients undergoing unselected cardiac surgery with cardioplegia*

Endpoint	Control (n=18) (mean [SD])	RIPC (n=23) (mean [SD])	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	31546.88 (17412.40)	35719.90 (26354.35)	-4712.13 (-19634.78, 11290.53)	0.587
Creatinine (mg/ml)				
Pre-operatively	91.4 (20.4)	92.5 (33.2)	-1.0 (-19.1, 17.0)	0.908
24 hours post-operatively	91.5 (25.9)	95.1 (34.6)	-3.6 (-23.6, 16.4)	0.718
48 hours post-operatively	104.1 (29.5)	102.8 (53.6)	1.3 (-27.2, 29.7)	0.928
72 hours post-operatively	105.6 (64.8)	100.9 (60.7)	4.6 (-35.2, 44.4)	0.816
Urine Output (ml)				
24 hours post-operatively	1817.5 (546.5)	1986.6 (559.1)	-169.1 (-535.4, 197.2)	0.355
48 hours post-operatively	2012.9 (868.9)	2217.3 (737.8)	-204.4 (-748.5, 339.8)	0.451
72 hours post-operatively	1771.3 (932.1)	2722.3 (910.4)	-951.1 (-1731.1, -171.0)	0.019
Total	5480.3 (1689.2)	6758.6 (1266.4)	-1278.3 (-2542.2, -14.3)	0.048
AKI score				
0	15 (83.3%)	22 (95.7%)		0.252
1	1 (5.6%)	1(4.3%)		
2	0 (0.0%)	0 (0.0%)		
3	2 (11.1%)	0 (0.0%)		
Acute Kidney Injury	2 (11.1%)	0 (0.0%)		0.101
Inotrope score				
Post bypass	6.81 (13.72)	5.46 (10.44)	1.35 (-6.49, 9.18)	0.729
24 hours post-operatively	10.17 (20.90)	8.491 (13.42)	1.68 (-9.48, 12.84)	0.762
48 hours post-operatively	7.88 (15.52)	5.76 (14.12)	2.13 (-7.52, 11.78)	0.658
72 hours post-operatively	5.86 (14.49)	0.57 (2.67)	5.29 (-2.22, 12.81)	0.156
Total	30.91 (53.45)	20.13 (33.57)	10.79 (-17.55, 39.12)	0.445
New onset AF	5 (27.8%)	3 (13.0%)		0.237
Length of ICU stay (days)	3.0 (2.0-5.0)**	2.0 (1.0-3.5)**		0.803***
Length of hospital stay (days)	9.0 (7.0–12.0)**	8.0 (6.0–10.5)**		0.207***
Clinical outcomes at six weeks				
Death	1 (5.6%)	0 (0.0%)		0.252
Myocardial infarction	0 (0.0%)	0 (0.0%)		1.000
Stroke	0 (0.0%)	0 (0.0%)		1.000
Revascularization	0 (0.0%)	0 (0.0%)		1.000

Table 6.10. Summary of major secondary endpoints in non-diabetic patients undergoing unselected cardiac surgery with cardioplegia*

Endpoint	Control (n=55) (mean [SD])	RIPC (n=52) (mean [SD])	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	29889.32 (11607.54)	35763.08 (24462.53)	-5873.766 (-14808.49, 3060.97)	0.193
Creatinine (mg/ml)				
Pre-operatively	85.3 (19.1)	80.9 (21.5)	4.3 (-3.5, 12.1)	0.273
24 hours post-operatively	88.2 (26.0)	83.9 (23.1)	4.3 (-5.2, 13.7)	0.374
48 hours post-operatively	95.1 (40.7)	85.4 (28.7)	9.7 (-3.9, 23.3)	0.160
72 hours post-operatively	90.7 (41.4)	85.8 (29.8)	4.9 (-9.02, 18.8)	0.489
Urine Output (ml)				
24 hours post-operatively	2060.0 (818.3)	2220.9 (542.6)	-160.9 (-438.4, 116.7)	0.253
48 hours post-operatively	2216.9 (945.5)	2351.7 (855.2)	-134.8 (-516.9, 247.3)	0.485
72 hours post-operatively	2013.3 (725.7)	2381.6 (860.8)	-368.2 (-776.8, 40.3)	0.076
Total	5969.7 (1721.2)	6659.4 (1776.8)	-689.7 (-1586.3, 207.0)	0.129
AKI score				
0	47 (85.5%)	49 (94.2%)		0.228
1	6 (10.9%)	3 (5.8%)		
2	2 (3.6%)	0 (0.0%)		
3	0 (0.0%)	0 (0.0%)		
Acute Kidney Injury	8 (14.5%)	3 (5.8%)		0.135
Inotrope score				
Post bypass	7.58 (14.02)	8.68 (18.34)	-1.10 (-7.46, 5.26)	0.732
24 hours post-operatively	12.49 (20.79)	10.40 (18.51)	2.10 (-5.65, 9.84)	0.593
48 hours post-operatively	6.85 (14.43)	6.09 (15.19)	0.76 (-5.09, 6.61)	0.796
72 hours post-operatively	3.35 (9.073)	1.92 (9.87)	1.42 (-2.32, 5.16)	0.452
Total	30.59 (48.22)	25.85 (47.87)	4.74 (-14.23, 23.71)	0.621
New onset AF	12 (21.8%)	6 (11.5%)		0.155
Length of ICU stay (days)	2.0 (2.0-4.0)**	2.0 (1.5-3.5)**		0.898***
Length of hospital stay (days)	9.0 (7.0–12.5)**	8.0 (6.0–10.5)**		0.176***
Clinical outcomes at six weeks				
Death	1 (1.8%)	0 (0.0%)		0.329
Myocardial infarction	1 (1.8%)	0 (0.0%)		0.329
Stroke	1 (1.8%)	0 (0.0%)		0.215
Revascularization	1 (1.8%)	0 (0.0%)		0.329

*List of abbreviations.
RIPC=Remote ischemic preconditioning; CK=Creatinine Kinase; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve. **Results shown as median (inter-quartile range); *** P-value for Mann-Whitney-Wilcoxon test

6.5. Effect of multi-limb RIPC on cardioprotection in diabetic and non-diabetic patients undergoing CABG surgery

Similarly to the subgroup analysis conducted in chapter 3, we intended to determine whether our enhanced preconditioning stimulus can protect diabetic and/or non/diabetic patients undergoing CABG surgery alone.

Within the diabetic sub-population, 17 subjects received the sham protocol and 19 non-diabetics the RIPC protocol, whereas in the non-diabetic group sham and RIPC patients were 37 and 38 respectively. Interestingly, control patients in the DM group had a lower mean diastolic BP ($p=0.036$), a lower mean HR ($p=0.030$), a reduced use of statins pre-operatively ($p=0.031$). In the non-DM group, the only significant difference between control and RIPC subjects was the lower incidence of PAD in the latter ($p=0.037$).

Table 6.11. Patient baseline characteristics in diabetic and non-diabetic patients undergoing CABG surgery

Patients	Diabetics			Non-diabetics		
	Control (n=17) (mean (SD))	RIPC (n=19) (mean (SD))	P value	Control (n=37) (mean (SD))	RIPC (n=38) (mean (SD))	P value
Age (*years*)	65±8	63±10	0.549	67±10	64±10	0.263
Gender			0.296			0.720
Male	13 (76.5%)	17 (89.5%)		30 (81.1%)	32 (84.2%)	
Female	4 (23.5%)	2 (10.5%)		7 (18.9%)	6 (15.8%)	
Ethnicity			0.466			0.740
Caucasian	12 (70.6%)	14 (73.7%)		31 (83.8%)	34 (89.5%)	
Asian	3 (17.6%)	5 (26.3%)		4 (10.8%)	3 (7.9%)	
Afro-Caribbean	1 (5.9%)	0 (0%)		2 (5.4%)	1 (2.6%)	
Chinese	1 (5.9%)	0 (0%)		0 (0%)	0 (0%)	
BMI	28.9±5.5	30.0±5.4	0.575	28.2±4.7	29.1±9.1	0.609
SBP *(mmHg)*	135.0±21	128.0±17	0.516	127.8±18.9	126.9±16.1	0.788
DBP *(mmHg)*	73.7±11	67.2±6.2	0.036	69.7±8.2	70.5±9.9	0.722
HR *(bpm)*	72.4±12.1	64.4±8.7	0.030	64.5±10.4	64.4±10.1	0.848
Smoking History			0.106			0.834
Smoker	4 (23.5%)	1 (5.3%)		5 (13.5%)	7 (18.4%)	
Ex-smoker	11 (64.7%)	11 (57.9%)		20 (54.1%)	20 (52.6%)	
Non-smoker	2 (11.8%)	7 (36.8%)		12 (32.4%)	11 (28.9%)	
Family History of IHD	11 (64.7%)	15 (78.9%)	0.341	28 (75.7%)	27 (71.1%)	0.651
NYHA Class	2.60±0.63	2.61±0.78	0.965	2.39±0.87	2.31±0.82	0.678
CCS Class	2.87±1.1	2.83±0.9	0.924	2.47±1.0	2.31±0.82	0.473
LVEF			0.606			0.721
>50%	10 (58.8%)	14 (73.7%)		13 (72.2%)	15 (65.2%)	
30%-50%	5 (29.4%)	4 (21.1%)		4 (22.2%)	5 (21.7%)	
<30%	2 (11.8%)	1 (5.3%)		1 (5.6%)	3 (13.0%)	
Co-morbidities						
Hypertension	15 (88.2%)	18 (94.7%)	0.481	30 (81.1%)	24 (63.2%)	0.084
Hypercholesterolemia	16 (94.1%)	18 (94.7%)	0.935	32 (86.5%)	28 (73.7%)	0.166
Atrial Fibrillation	1 (5.9%)	1 (5.3%)	0.935	4 (10.8%)	3 (7.9%)	0.502
Previous MI	4 (23.5%)	7 (36.8%)	0.387	18 (48.6%)	15 (39.5%)	0.424
Previous PCI	5 (29.4%)	5 (26.3%)	0.836	4 (10.8%)	4 (10.5%)	0.968
Previous CVA/TIA	2 (11.8%)	1 (5.3%)	0.558	4 (10.8%)	2 (5.3%)	0.178
Previous Cardiac Surgery	0 (0.0%)	0 (0.0%)	1.000	0 (0.0%)	1 (2.6%)	0.321
Other comorbidities	1 (5.9%)	1 (3.9%)	0.837	3 (8.1%)	1 (2.6%)	0.213
Peripheral Arterial Disease	2 (11.8%)	1 (5.3%)	0.917	4 (10.8%)	0 (0.0%)	0.037
Drug History						
Aspirin	13 (86.6%)	17 (89.5%)	0.670	33 (89.2%)	36 (100.0%)	0.127
Clopidogrel/Prasugrel	5 (33.3%)	9 (47.7%)	0.409	16 (43.3%)	10 (27.8%)	0.183
Warfarin	1 (6.7%)	2 (10.5%)	0.239	2 (5.4%)	0 (0.0%)	0.157
Beta-blocker	12 (80.0%)	16 (82.2%)	0.749	29 (78.4%)	24 (66.7%)	0.262
Calcium Channel Blocker	6 (40.0%)	4 (21.1%)	0.212	14 (37.8%)	8 (22.2%)	0.263
Statin	16 (83.3%)	18 (84.7%)	0.031	35 (94.6%)	34 (88.9%)	0.446
ACE-I/ARB	12 (80.0%)	15 (79.3%)	0.968	24 (63.9%)	23 (63.9%)	0.863
Long acting nitrates	7 (46.7%)	5 (26.3%)	0.218	6 (14.2%)	6 (16.7%)	0.579
Antidiabetics						
Insulin	6 (40.0%)	7 (36.8%)	0.851			
Biguanide	6 (40.0%)	10 (62.6%)	0.564			
Sulphonylurea	7 (46.7%)	4 (21.1%)	0.116			
Diuretics	6 (40.0%)	6 (31.6%)	0.843	7 (18.9%)	9 (25.0%)	0.724

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA=Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF=left ventricular ejection fraction.

Table 6.12. Details of surgical procedure in diabetic and non-diabetic patients undergoing CABG surgery

Patients	Diabetics			Non-diabetics		
	Control (n=17) (mean (SD))	RIPC (n=19) (mean (SD))	P value	Control (n=37) (mean (SD))	RIPC (n=38) (mean (SD))	P value
Indication for Surgery			0.023			0.611
Angina	17 (100.0%)	14 (73.7%)		26 (70.3%)	26 (39.1%)	
Myocardial Infarction	0 (0.0%)	5 (26.3%)		11 (29.7%)	27 (71.0%)	
EuroSCORE	2.76±1.9	2.68±2.9	0.923	3.35±1.8	2.92±2.1	0.346
Additive perioperative risk			0.640			0.815
Low (EuroSCORE 0-2)	9 (52.9%)	9 (47.4%)		13 (35.1%)	16 (42.1%)	
Medium (EuroSCORE 3-5)	7 (41.2%)	7 (36.8%)		19 (51.4%)	17 (44.7%)	
High (EuroSCORE >5)	1 (5.9%)	3 (15.8%)		5 (13.5%)	5 (13.2%)	
Bypass-time (min)	91.47±25.72	83.05±20.21	0.293	84.86±30.45	85.74±22.77	0.888
Cross-clamp time (min)	51.93±16.34	56.79±18.99	0.461	56.24±25.37	53.87±19.18	0.648
Myocardial Preservation						0.739
Cardioplegia	11 (64.7%)	14 (73.7%)	0.090	26 (73.0%)	29 (76.3%)	
Cross-clamp Fibrillation	6 (35.3%)	5 (26.3%)		10 (27.0%)	9 (23.7%)	
Number of grafts						0.987
One	0 (0.0%%)	0 (0.0%%)	0.527	1 (2.7%)	1 (2.6%)	
Two	5 (29.4%)	5 (26.3%)		8 (21.6%)	4 (17.4%)	
Three	7 (41.2%)	11 (57.9%)		21 (56.8%)	3 (13.0%)	
Four	5 (29.4%)	3 (15.8%)		7 (18.9%)	4 (17.4%)	
Anesthetic agents						
Induction						
Anti-nicotinic agents			0.831			0.218
Rocuronium	13 (86.7%)	15 (83.3%)		29 (82.9%)	34 (94.4%)	
Pancuronium	1 (5.9%)	2 (11.2%)		5 (14.3%)	1 (2.8%)	
Vecuronium	1 (5.9%)	1 (5.6%)		1 (2.9%)	1 (2.8%)	
Midazolam	5 (33.3%)	9 (50.0%)	0.335	9 (50.0%)	9 (40.9%)	0.076
Etomidate	0 (0.0%)	3 (16.7%)	0.097	20 (57.1%)	13 (36.1%)	0.233
Fentanyl	17 (100.0%)	18 (100.0%)	1.000	5 (13.9%)	2 (5.6%)	1.000
Propofol	17 (100.0%)	18 (100.0%)	1.000	37 (100%)	38 (100%)	0.453
Maintenance						
Propofol	17 (100.0%)	18 (100.0%)	1.000	36 (100%)	38 (100%)	1.000
Volatile Anesthetics						0.555
Isoflurane	13 (13.3%)	16 (88.9%)	0.846	34 (94.4%)	35 (97.2%)	
Sevoflurane	2 (13.3%)	2 (11.1%)	0.846	2 (5.6%)	1 (2.8%)	
Intra-operative GTN	15 (88.2%)	13 (68.4%)	0.153	31 (88.6%)	28 (75.7%)	0.155

RIPC=Remote Ischemic Preconditioning; CABG=Coronary artery bypass graft; AVR=Aortic valve replacement; MVR=Mitral valve replacement; MV=Mitral valve.

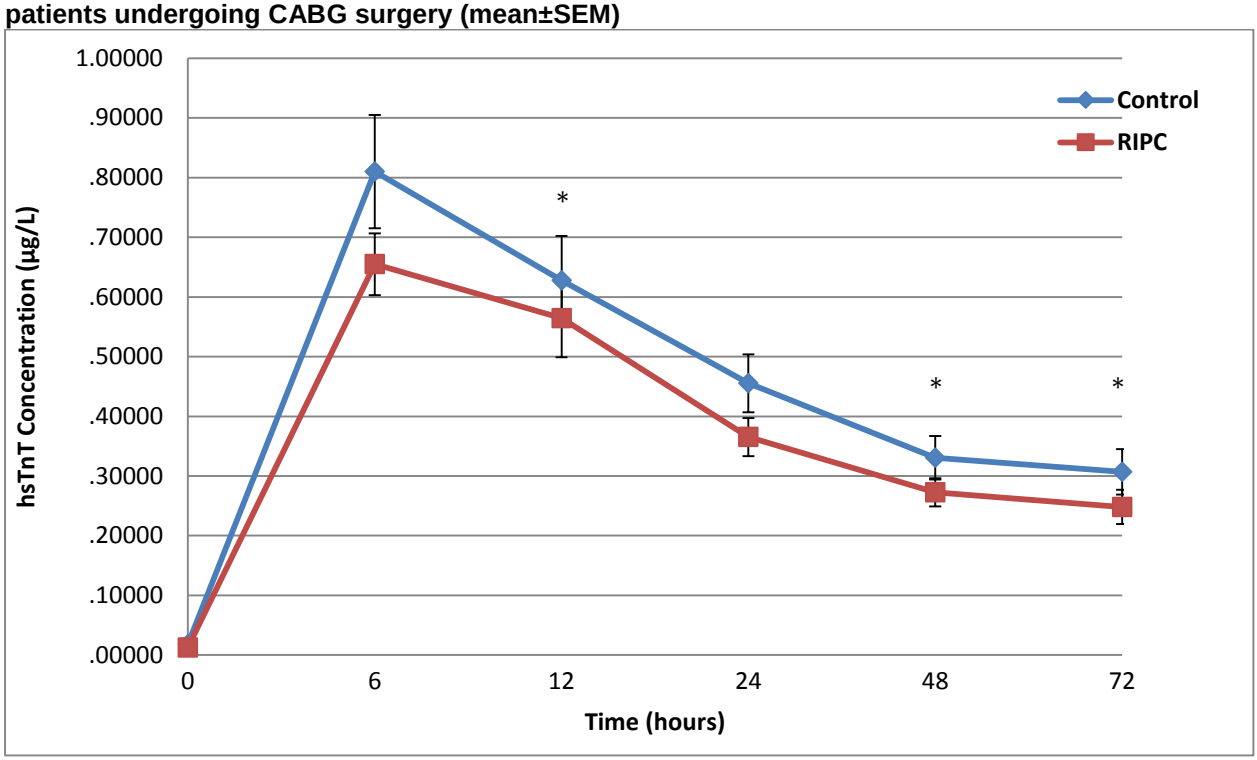
Intriguingly, we found that in diabetic patients RIPC significantly reduced mean hsTnT at 12, 48 and 72 hours **(Fig. 6.7, Table 6.13)** and total AUC from 31.73±18.63 μg/L to 19.63±9.19 μg/L, which corresponded to a statistically significant reduction of 38% [12.09; CI 1.83, 22.35; p=0.022] **(Fig. 6.9, Table 6.13)**. Conversely, in non-diabetic subjects no significant reduction of hsTnT concentrations or total AUC was observed **(Figs. 6.8-6.9, Table 6.13)**.

Table 6.13. Mean high-sensitivity Troponin T release at the specified time points in diabetic and non-diabetic patients undergoing CABG surgery

Endpoint		**Control** **DM: n=17** **Non-DM: n=37** **(mean (sd))**	**RIPC** **DM: n=19** **Non-DM: n=38** **(mean (sd))**	**Difference** **(95% CI)**	**P value**
hsTnT *(μg/L)*					
Pre-operatively	DM	0.024 (0.025)	0.016 (0.025)	0.008 (-0.009, 0.026)	0.330
	No-DM	0.018 (0.017)	0.012 (0.018)	-0.006 (-0.002, 0.013)	0.150
6 hours post-operatively	DM	0.709 (0.328)	0.544 (0.302)	0.164 (-0.049, 0.378)	0.126
	No-DM	0.809 (0.576)	0.655 (0.318)	0.155 (-0.061, 0.371)	0.156
12 hours post-operatively	DM	0.653 (0.276)	0.459 (0.211)	0.194 (0.029, 0.359)	0.023
	No-DM	0.628 (0.455)	0.564 (0.404)	0.063 (-0.134, 0.261)	0.527
24 hours post-operatively	DM	0.515 (0.283)	0.302 (0.145)	0.147 (-0.009, 0.305)	0.065
	No-DM	0.455 (0.295)	0.365 (0.196)	0.090 (-0.026, 0.206)	0.125
48 hours post-operatively	DM	0.389 (0.322)	0.217 (0.101)	0.173 (0.011, 0.335)	0.038
	No-DM	0.331 (0.218)	0.272 (0.146)	0.058 (-0.029, 0.144)	0.187
72 hours post-operatively	DM	0.368 (0.251)	0.158 (0.088)	0.210 (0.079, 0.342)	0.004
	No-DM	0.307 (0.228)	0.307 (0.174)	0.06 (-0.036, 0.154)	0.218
Total 72 hours AUC	DM	31.73 (18.63)	19.63 (9.19)	12.09 (1.83, 22.35)	0.022
	No-DM	30.29 (19.34)	25.43 (12.79)	4.86 (-2.84, 12.56)	0.212

RIPC=Remote ischemic preconditioning; SD=standard deviation; CI=confidence interval; hsTnT=high sensitivity Troponin T

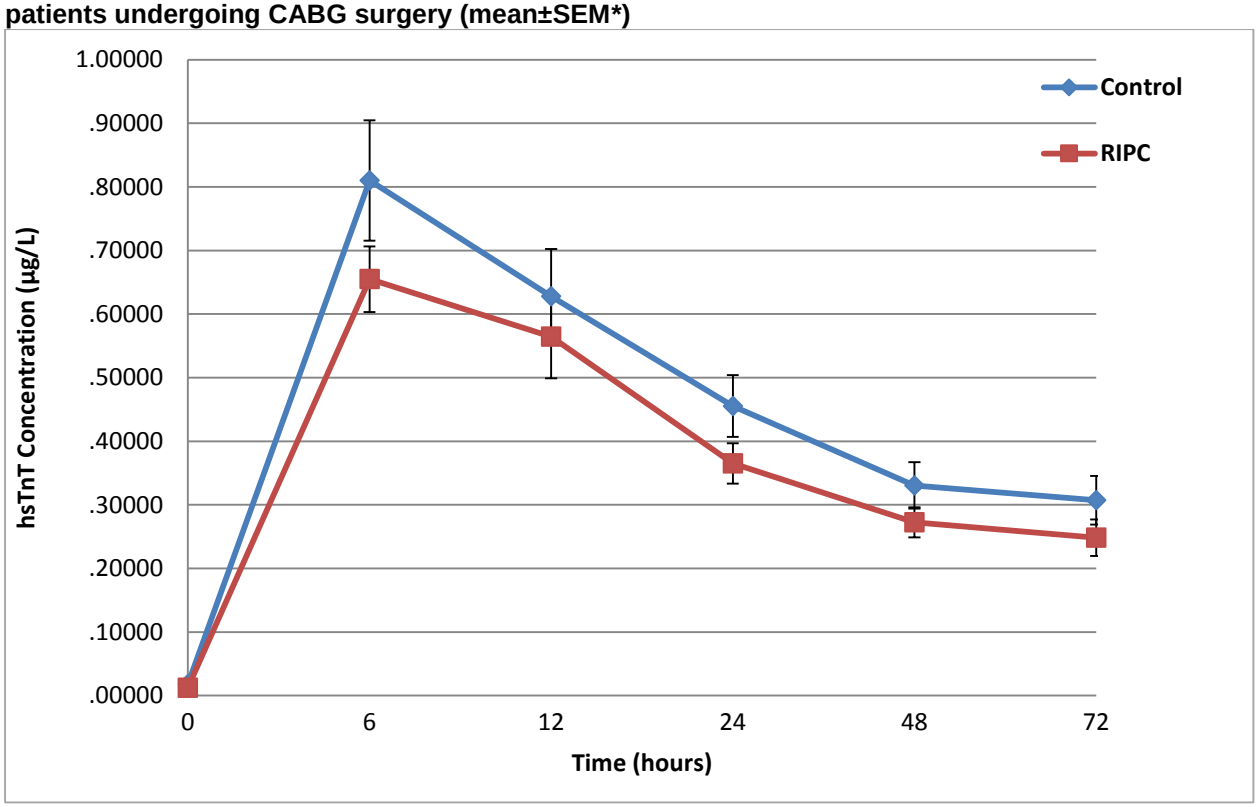
Fig. 6.7. Mean high-sensitivity Troponin-T levels at 0, 6, 12, 24, 48 and 72 hours in diabetic patients undergoing CABG surgery (mean±SEM)



RIPC=remote ischaemic preconditioning; hsTnT=high sensitivity troponin-T; SEM=standard error of the mean

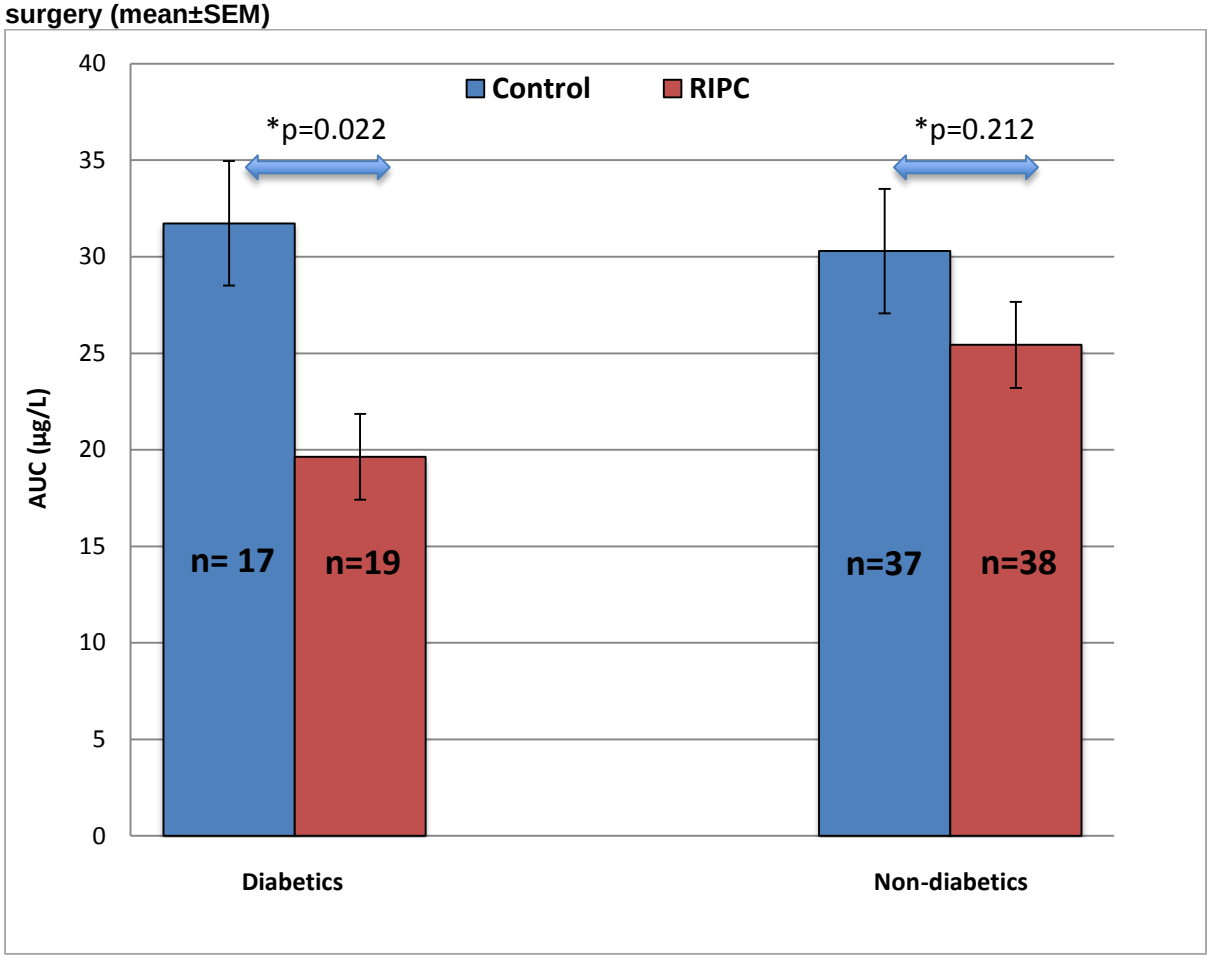
• p<0.05 (unpaired Student T-Test)

Fig. 6.8. Mean high-sensitivity Troponin-T levels at 0, 6, 12, 24, 48 and 72 hours in non-diabetic patients undergoing CABG surgery (mean±SEM*)



RIPC=remote ischaemic preconditioning; hsTnT=high sensitivity troponin-T; SEM=standard error of the mean. * Unpaired Student T-Test

Fig. 6.9. Total AUC in control and RIPC diabetic and non-diabetic patients undergoing CABG surgery (mean±SEM)



RIPC=remote ischaemic preconditioning; SEM=standard error of the mean; AUC=area-under-the-curve
* Unpaired Student T-Test

Additionally, we found that simultaneous multi-limb preconditioning significantly reduced the incidence of new post-operative AF of 83% in the diabetic cohort, with only 1 new case of AF in the RIPC group versus 6 new cases amongst control (p=0.023) **(Table 6.14)**. In the non-diabetic group, the only significant difference between the two intervention cohorts was given by the improvement in urine output at 24 hours in preconditioned patients (p=0.041) **(Table 6.15)**. Interestingly, we found no death, revascularisation, stroke or MI at 6 weeks in any of the above-mentioned subgroups **(Tables 6.14-6.15)**.

Table 6.14. Summary of study endpoints in diabetic patients undergoing CABG surgery*

Endpoint	Control (n=18) (mean [SD])	RIPC (n=23) (mean [SD])	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	30348.50 (19176.29)	30933.80 (20762.26)	-585.30 (-16602.13, 15431.53)	0.941
Creatinine (mg/ml)				
Pre-operatively	93.9 (20.14)	94.4 (32.5)	-0.5 (-19.1, 17.9)	0.953
24 hours post-operatively	103.4 (41.1)	93.3 (28.3)	10.1 (-14.0, 34.3)	0.399
48 hours post-operatively	118.1 (51.4)	102.2 (39.6)	15.9 (-14.9, 46.8)	0.303
72 hours post-operatively	109.9 (39.5)	99.4 (19.8)	10.5 (-18.4, 39.4)	0.465
Urine Output (ml)				
24 hours post-operatively	1931.6 (788.6)	2022.6 (864.7)	-91.0 (-704.8, 522.7)	0.764
48 hours post-operatively	2164.7 (961.9)	2279.6 (884.3)	-114.9 (-835.5, 605.7)	0.746
72 hours post-operatively	2136.6 (1077.9)	2632.5 (926.7)	-495.9 (-1466.0, 474.2)	0.296
Total	5776.1 (1723.9)	6488.2 (1410.4)	-712.1 (-229.9, 805.7)	0.336
AKI score				
0	13 (76.5%)	18 (94.7%)		0.386
1	2 (11.8%)	1(5.3%)		
2	1 (5.9%)	0 (0.0%)		
3	1 (5.9%)	0 (0.0%)		
Acute Kidney Injury	4 (23.5%)	1 (5.3%)		0.114
Inotrope score				
Post bypass	6.28 (14.87)	2.87 (8.05)	3.41 (-4.97, 11.79)	0.412
24 hours post-operatively	11.10 (22.77)	5.82 (9.93)	5.28 (-6.91, 17.47)	0.383
48 hours post-operatively	15.64 (26.38)	0.98 (2.49)	14.66 (1.95, 27.37)	0.058
72 hours post-operatively	8.44 (21.72)	0.17 (0.71)	8.28 (-2.14, 18.68)	0.178
Total	41.62 (66.46)	9.83 (15.39)	31.79 (-7.07, 70.65)	0.101
New onset AF	6 (35.3%)	1 (5.3%)		0.023
Length of ICU stay (days)	2.5 (2.0-5.5)**	1.0 (1.0-2.0)**		0.085***
Length of hospital stay (days)	9.0 (7.0-14.5)**	8.0 (6.0-9.0)**		0.128***
Clinical outcomes at six weeks				
Death	0 (0.0%)	0 (0.0%)		1.000
Myocardial infarction	0 (0.0%)	0 (0.0%)		1.000
Stroke	0 (0.0%)	0 (0.0%)		1.000
Revascularization	0 (0.0%)	0 (0.0%)		1.000

Table 6.15. Summary of study endpoints in non-diabetic patients undergoing CABG surgery*

Endpoint	Control (n=18) (mean [SD])	RIPC (n=23) (mean [SD])	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	36494.22 (26109.06)	37767.12 (30831.17)	-1272.90 (-17810.34, 15264.54)	0.876
Creatinine (mg/ml)				
Pre-operatively	86.7 (18.9)	82.0 (19.9)	-4.7 (-4.2, 13.7)	0.296
24 hours post-operatively	91.5 (25.9)	95.1 (34.6)	6.9 (-4.2, 17.9)	0.220
48 hours post-operatively	104.1 (29.5)	102.8 (53.6)	18.2 (-1.5, 37.9)	0.070
72 hours post-operatively	105.6 (64.8)	100.9 (60.7)	11.2 (-7.9, 30.4)	0.248
Urine Output (ml)				
24 hours post-operatively	1981.6 (546.5)	1986.6 (590.6)	-297.3.1 (-581.7, -12.8)	0.041
48 hours post-operatively	2224.5 (1050.9)	2298.2 (773.7)	-73.7 (-555.2, 407.7)	0.760
72 hours post-operatively	2116.4 (898.6)	2301.9 (728.7)	-185.5 (-686.5, 315.4)	0.459
Total	6109.9 (2171.5)	6576.6 (1391.0)	-466.7 (-1568.3, 634.9)	0.397
AKI score				
0	32 (86.5%)	36 (94.7%)		0.528
1	3 (8.1%)	1(2.6%)		
2	1 (2.7%)	1(2.6%)		
3	1 (2.7%)	0 (0.0%)		
Acute Kidney Injury	5 (13.5%)	2 5.2%)		0.219
Inotrope score				
Post bypass	5.44 (10.86)	7.24 (17.82)	-1.79 (-8.81, 5.22)	0.611
24 hours post-operatively	9.31 (18.71)	9.47 (18.40)	-0.15 (-9.01, 8.70)	0.972
48 hours post-operatively	6.80 (18.92)	5.94 (16.29)	0.86 (-7.60, 9.33)	0.839
72 hours post-operatively	4.08 (17.69)	1.44 (5.44)	2.64 (-3.54, 8.82)	0.397
Total	25.85 (61.41)	24.08 (48.82)	1.76 (-24.79, 28.31)	0.895
New onset AF	9 (24.3%)	5 (13.2%)		0.215
Length of ICU stay (days)	2.0 (1.0-4.0)**	2.0 (1.0–4.0)**		0.376***
Length of hospital stay (days)	8.0 (6.0–11.0)**	7.0 (6.0–9.0)**		0.276***
Clinical outcomes at six weeks				
Death	0 (0.0%)	0 (0.0%)		1.000
Myocardial infarction	0 (0.0%)	0 (0.0%)		1.000
Stroke	0 (0.0%)	0 (0.0%)		1.000
Revascularization	0 (0.0%)	0 (0.0%)		1.000

*List of abbreviations.
RIPC=Remote ischemic preconditioning; CK=Creatinine Kinase; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve. **Results shown as median (inter-quartile range); *** P-value for Mann-Whitney-Wilcoxon test

6.6. Effect of multi-limb RIPC on cardioprotection in diabetic and non-diabetic patients undergoing CABG surgery using cardioplegia

In our previous analysis, we found that our enhanced preconditioning stimulus protected diabetic patients undergoing CABG surgery alone. However, we could not find similar beneficial effects in a similar cohort of non-diabetic patients. Moreover, in chapter 3 we found that the same stimulus improved cardioprotection in both CABG patients alone and in subjects undergoing cardiac surgery with cardioplegia but not in CABG patients receiving cardioplegia. We therefore wished to conduct a further subgroup analysis to determine whether multi-limb IR reduced PMI in diabetic and/or non-diabetic patients undergoing CABG surgery and receiving cardioplegia.

The diabetic subgroup comprised 11 control patients and 14 RIPC subjects, whereas the non-diabetic subgroup had 27 controls and 29 preconditioned subjects **(Table 6.16)**. Diabetic control patients had a more significant non-smoking history ($p=0.027$): we found no other significant difference amongst the 4 subgroups **(Table 6.16-6.17)**.

Table 6.16. Patient baseline characteristics in diabetic and non-diabetic patients undergoing CABG surgery with cardioplegia

Patients	Diabetics			Non-diabetics		
	Control (n=11) (mean (SD))	RIPC (n=14) (mean (SD))	P value	Control (n=27) (mean (SD))	RIPC (n=29) (mean (SD))	P value
Age (*years*)	66±6	66±11	0.952	69±9	65±9	0.138
Gender			0.792			0.838
Male	9 (81.8%)	12 (85.7%)		22 (81.5%)	23 (79.3%)	
Female	2 (18.2%)	2 (14.3%)		5 (18.5%)	6 (20.7%)	
Ethnicity			0.411			0.994
Caucasian	7 (63.6%)	10 (71.4%)		23 (85.2%)	25 (86.2%)	
Asian	2 (18.2%)	4 (28.6%)		3 (11.1%)	3 (10.3%)	
Afro-Caribbean	1 (9.1%)	0 (0%)		1 (3.7%)	1 (3.4%)	
Chinese	1 (9.1%)	0 (0%)		0 (0%)	0 (0%)	
BMI	29.0±5.0	29.4±4.9	0.901	27.1±4.7	29.4±10.3	0.298
SBP *(mmHg)*	136.4±20	129.6±17	0.381	128.3±20.6	126.9±18.3	0.796
DBP *(mmHg)*	72.1±9	67.9±6.1	0.181	69.1±8.4	70.0±10.7	0.724
HR *(bpm)*	69.6±12.1	61.4±6.5	0.064	65.3±11.5	65.2±10.4	0.978
Smoking History			0.027			0.203
Smoker	3 (27.3%)	0 (0%)		2 (7.4%)	6 (20.7%)	
Ex-smoker	7 (63.6%)	7 (50.0%)		16 (59.3%)	18 (62.1%)	
Non-smoker	1 (9.1%)	7 (50.0%)		9 (33.3%)	5 (17.2%)	
Family History of IHD	8 (72.7%)	10 (71.4%)	0.943	18 (66.7%)	22 (75.9%)	0.447
NYHA Class	2.73±0.48	2.77±0.73	0.871 0.695	2.50±1.11	2.57±0.99	0.658
CCS Class	3.00±1.0	2.85±0.9	0.672	2.47±1.0	2.31±0.82	0.804
LVEF						0.362
>50%	6 (54.5%)	10 (71.4%)		20 (74.1%)	21 (72.4%)	
30%-50%	4 (36.4%)	3 (21.4%)		7 (25.9%)	6 (20.7%)	
<30%	1 (9.1%)	1 (7.1%)		0 (0.0%)	2 (6.9%)	
Co-morbidities			0.859			
Hypertension	10 (90.9%)	13 (92.9%)	0.366	22 (81.5%)	20 (69.0%)	0.280
Hypercholesterolemia	11 (100.0%)	13 (92.9%)	0.250	22 (81.5%)	21 (72.4%)	0.422
Atrial Fibrillation	1 (9.1%)	0 (0.0%)	0.973	3 (11.1%)	1 (3.4%)	0.485
Previous MI	4 (36.4%)	5 (35.7%)	0.678	15 (55.6%)	12 (41.4%)	0.289
Previous PCI	4 (36.4%)	4 (28.6%)	0.500	4 (14.8%)	2 (6.9%)	0.338
Previous CVA/TIA	2 (18.2%)	1 (7.1%)	1.000	4 (14.8%)	2 (6.9%)	0.166
Previous Cardiac Surgery	0 (0.0%)	0 (0.0%)	0.918	0 (0.0%)	1 (3.4%)	0.330
Other comorbidities	1 (9.1%)	1 (7.1%)	0.399	2 (7.4%)	1 (3.4%)	0.213
Peripheral Arterial Disease	2 (18.2%)	1 (7.1%)		3 (11.1%)	0 (0.0%)	0.461
Drug History			0.916			
Aspirin	10 (89.9%)	12 (85.7%)	0.821	24 (88.9%)	29 (100.0%)	0.190
Clopidogrel/Prasugrel	5 (45.5%)	7 (50.0%)	0.357	13 (48.1%)	8 (28.6%)	0.175
Warfarin	1 (9.1%)	1 (7.1%)	0.840	2 (7.4%)	0 (0.0%)	0.142
Beta-blocker	9 (81.8%)	11 (78.6%)	0.179	21 (77.8%)	17 (60.7%)	0.171
Calcium Channel Blocker	6 (54.6%)	3 (21.4%)	0.694	12 (44.4%)	7 (25.0%)	0.238
Statin	11 (100.0%)	14 (100.0%)	0.482	24 (92.6%)	26 (89.3%)	0.493
ACE-I/ARB	10 (81.9%)	10 (71.4%)	0.346	16 (59.3%)	19 (67.9%)	0.474
Long acting nitrates	6 (54.6%)	5 (35.7%)		5 (18.5%)	6 (14.3%)	0.586
Antidiabetics						
Insulin	4 (36.4%)	4 (28.6%)	0.678			
Biguanide	4 (36.4%)	10 (71.4%)	0.773			
Sulphonylurea	10 (91.0%)	14 (100.0%)	0.293			
Diuretics	66 (54.6%)	3 (21.4%)	0.196	3 (11.1%)	7 (25.0%)	0.321

RIPC=Remote Ischemic Preconditioning; NYHA=New York Health Association; CCS=Canadian Cardiovascular Society; MI=Myocardial infarction; PCI=Percutaneous coronary intervention; CVA=Cerebrovascular accident; TIA=Transient ischemic attack; ACE-I=Angiotensin-converting enzyme-inhibitor; ARB=Angiotensin receptor blocker; LVEF=left ventricular ejection fraction.

Table 6.17. Details of surgical procedure in diabetic and non-diabetic patients undergoing CABG surgery with cardioplegia

Patients	Diabetics			Non-diabetics		
	Control (n=11)	RIPC (n=14)	P value	Control (n=27)	RIPC (n=29)	P value
Indication for Surgery			0.207			0.610
Angina	11 (100.0%)	9(64.3%)		19 (70.4%)	19 (65.5%)	
Myocardial Infarction	0 (0.0%)	5 (35.7%)		8 (29.6%)	9 (31.0%)	
EuroSCORE	2.76±1.9	2.68±2.9	0.566	3.63±1.76	3.03±2.04	0.249
Additive perioperative risk			0.367			0.621
Low (EuroSCORE 0-2)	7 (63.6%)	5 (35.7%)		7 (25.9%)	11 (37.9%)	
Medium (EuroSCORE 3-5)	3 (27.3%)	6 (42.9%)		16 (59.3%)	14 (48.3%)	
High (EuroSCORE >5)	1 (9.1%)	3 (21.4%)		4 (14.8%)	4 (13.8%)	
Bypass-time (min)	91.47±25.72	83.05±20.21	0.293	84.86±30.45	85.74±22.77	0.888
Cross-clamp time (min)	51.93±16.34	56.79±18.99	0.461	56.24±25.37	53.87±19.18	0.648
Number of grafts			0.793			0.810
One	0 (0.0%%)	0 (0.0%%)		1 (3.8%)	1 (3.6%)	
Two	4 (36.4%)	4 (28.6%)		6 (22.2%)	9 (31.0%)	
Three	4 (36.4%)	7 (50.0%)		14 (51.9%)	15 (51.7%)	
Four	3 (27.3%)	3 (21.4%)		6 (22.2%)	4 (13.8%)	
Anesthetic agents						
Induction						
Anti-nicotinic agents			0.983			0.218
Rocuronium	9 (81.8%)	11 (84.6%)		21 (80.8%)	27 (96.4%)	
Pancuronium	1 (9.1%)	1 (7.7%)		4 (15.4%)	1 (3.6%)	
Vecuronium	1 (9.1%)	1 (7.7%)		1 (3.8%)	0 (0.0%)	
Midazolam	5 (45.5%)	6 (46.2%)	0.973	13 (50.0%)	11(39.3%)	0.429
Etomidate	0 (0.0%)	3 (23.1%)	0.089	3 (11.5%)	2 (7.1%)	0.233
Fentanyl	11 (100.0%)	14 (100.0%)	1.000	27 (100%)	29 (100%)	1.000
Propofol	11 (100.0%)	11 (76.9%)	0.089	23 (88.5%)	25 (89.3%)	0.923
Maintenance						
Propofol	11 (100.0%)	14 (100.0%)	1.000	27 (100%)	29 (100%)	1.000
Volatile Anesthetics						0.295
Isoflurane	8 (81.8%)	12 (92.3%)	0.439	25 (96.2%)	29 (100%)	
Sevoflurane	2 (18.2%)	1 (7.7%)		1 (3.8%)	0 (0.0%)	
Intra-operative GTN	10 (90.9%)	8 (57.1%)	0.062	22 (88.0%)	21(75.0%)	0.227

RIPC=Remote Ischemic Preconditioning; CABG=Coronary artery bypass graft; AVR=Aortic valve replacement; MVR=Mitral valve replacement; MV=Mitral valve.

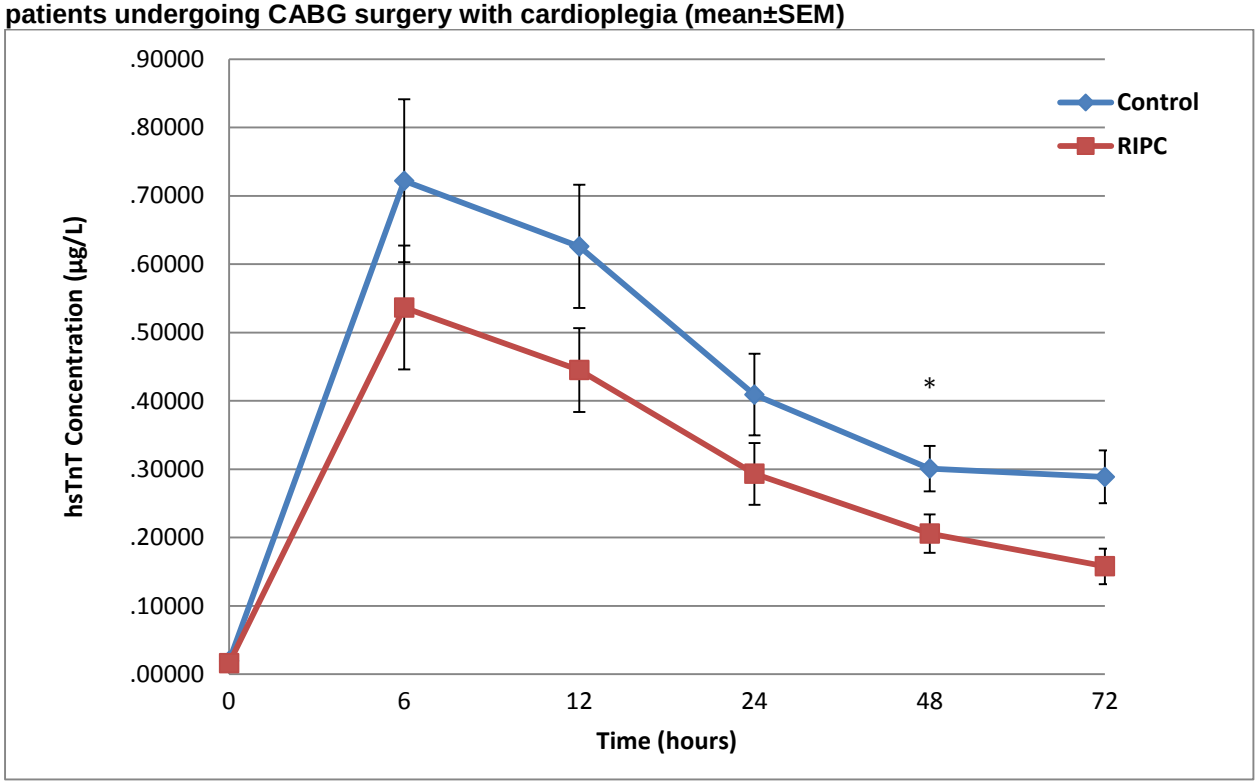
Interestingly we found that mean hsTnT was reduced in preconditioned patients at 48 and 72 hours post-operatively in the DM group with no significant difference between sham and RIPC subjects in non-DM group **(Figs. 6.10-6.11, Table 6.18)**. Total AUC was reduced in preconditioned diabetics and non-diabetics although without reaching statistical relevance (respectively 27.59±11.46 μg/L versus 19.37±10.03 μg/L [8.22; CI -0.68, 17.11; p=0.069] and 30.78±21.81 μg/L versus 26.76±13.79 μg/L [4.02; CI -5.74, 13.78; p=0.413]) **(Fig. 6.12, Table 6.18)**.

Table 6.18. Mean high-sensitivity Troponin-T release in diabetic and non-diabetic patients undergoing CABG surgery with cardioplegia

Endpoint		Control DM: n=11 Non-DM: n=14 (mean (sd))	RIPC DM: n=27 Non-DM: n=29 (mean (sd))	Difference (95% CI)	P value
hsTnT *(μg/L)*					
Pre-operatively	DM	0.019 (0.022)	0.016 (0.028)	0.004 (-0.018, 0.025)	0.723
	No-DM	0.019 (0.017)	0.013 (0.019)	0.006 (-0.004, 0.015)	0.263
6 hours post-operatively	DM	0.722 (0.395)	0.537 (0.339)	0.185 (-0.119, 0.489)	0.220
	No-DM	0.849 (0.654)	0.709 (0.329)	0.141 (-0.142, 0.424)	0.320
12 hours post-operatively	DM	0.626 (0.299)	0.445 (0.229)	0.181 (0.037, 0.399)	0.100
	No-DM	0.642 (0.526)	0.605 (0.444)	0.038 (-0.222, 0.298)	0.771
24 hours post-operatively	DM	0.409 (0.197)	0.293 (0.169)	0.116 (-0.036, 0.268)	0.127
	No-DM	0.458 (0.326)	0.386 (0.211)	0.073 (-0.076, 0.222)	0.329
48 hours post-operatively	DM	0.301 (0.110)	0.206 (0.105)	0.095 (0.005, 0.184)	0.038
	No-DM	0.332 (0.239)	0.289 (0.158)	0.043 (-0.069, 0.154)	0.444
72 hours post-operatively	DM	0.289 (0.128)	0.158 (0.098)	0.131 (0.038, 0.224)	0.008
	No-DM	0.309 (0.248)	0.261 (0.192)	0.049 (-0.071, 0.168)	0.422
Total 72 hours AUC	DM	27.59 (11.46)	19.37 (10.03)	8.22 (-0.68, 17.11)	0.069
	No-DM	30.78 (21.81)	26.76 (13.79)	4.02 (-5.74, 13.78)	0.413

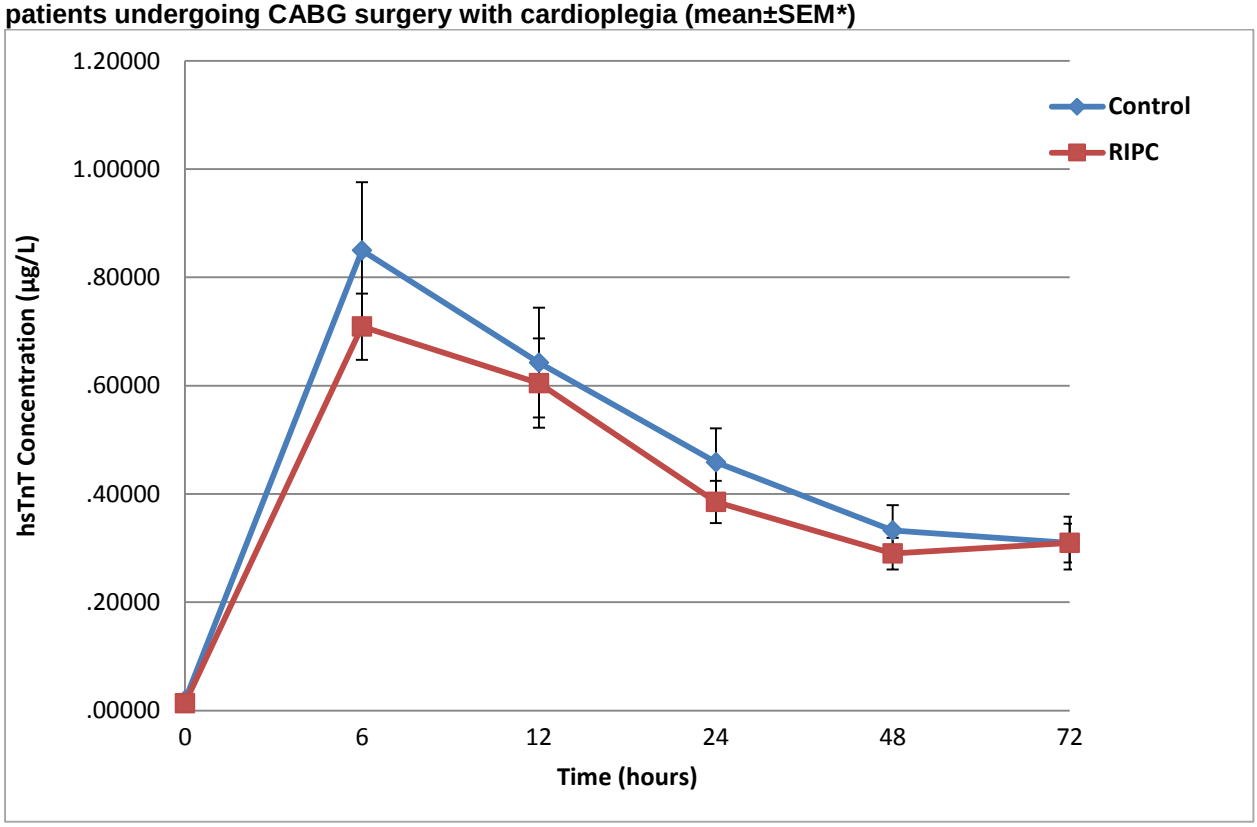
RIPC=Remote ischemic preconditioning; SD=standard deviation; CI=confidence interval; hsTnT=high sensitivity Troponin-T

Fig. 6.10. Mean high-sensitivity Troponin-T levels at 0, 6, 12, 24, 48 and 72 hours in diabetic patients undergoing CABG surgery with cardioplegia (mean±SEM)



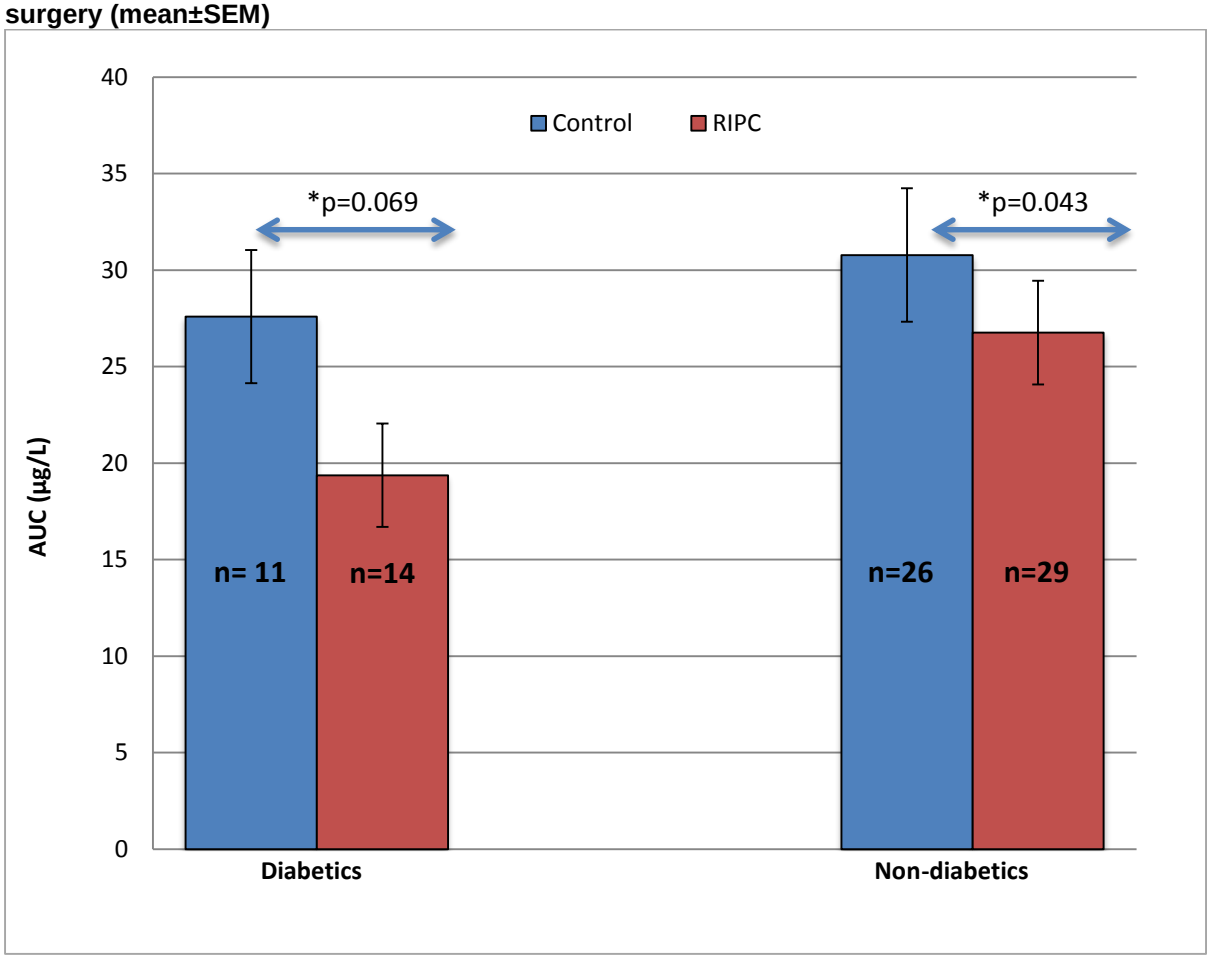
RIPC=remote ischaemic preconditioning; hsTnT=high sensitivity troponin-T; SEM=standard error of the mean. *p<0.05 (unpaired Student T-Test)

Fig. 6.11. Mean high-sensitivity Troponin-T levels at 0, 6, 12, 24, 48 and 72 hours in non-diabetic patients undergoing CABG surgery with cardioplegia (mean±SEM*)



RIPC=remote ischaemic preconditioning; hsTnT=high sensitivity troponin-T; SEM=standard error of the mean. *p<0.05 (unpaired Student T-Test)

Fig. 6.12. Total AUC in control and RIPC diabetic and non-diabetic patients undergoing CABG surgery (mean±SEM)



RIPC=remote ischaemic preconditioning; SEM=standard error of the mean; AUC=area-under-the-curve
* Unpaired Student T-Test

Amongst secondary endpoints, we observed a reduction of post-operative AKI and AF incidence in RIPC diabetic patients from 2 to 0 and from 4 to 1 respectively, which however only approximated to but did not reach statistical significance (p=0.096 and 0.070 respectively) **(Tables 6.19-6.20)**. No difference in any other secondary endpoint was found in this subgroup analysis **(Tables 6.19-6.20)**.

Table 6.19. Summary of study endpoints in diabetic patients undergoing CABG surgery with cardioplegia*

Endpoint	Control (n=11) (mean [SD])	RIPC (n=14) (mean [SD])	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	31624.64 (19570.55)	31319.77 (22243.63)	304.88 (-17401.82, 18011.55)	0.972
Creatinine (mg/ml)				
Pre-operatively	92.6 (17.3)	87.8 (21.6)	4.8 (11.8, 21.3)	0.557
24 hours post-operatively	96.2 (28.5)	89.7 (26.9)	6.5 (-17.0, 29.9)	0.573
48 hours post-operatively	107.3 (26.0)	90.9 (30.4)	16.4 (-7.4, 40.2)	0.167
72 hours post-operatively	97.6 (24.0)	86.9(25.5)	10.8 (-9.9, 31.5)	0.293
Urine Output (ml)				
24 hours post-operatively	1857.3 (671.5)	2035.3 (712.1)	-178.0 (-797.9, 441.9)	0.556
48 hours post-operatively	2153.3 (972.0)	2002.3 (884.3)	151.0 (-515.9, 817.9)	0.641
72 hours post-operatively	1914.6 (906.3)	2590.0 (1011.5)	-675.4 (-1795.1, 444.3)	0.213
Total	5664.4 (1807.7)	6404.5 (1053.1)	-740.1 (-2550.9, 1070.7)	0.391
AKI score				
0	9 (81.8%)	14 (100.0%)		0.251
1	1 (9.1%)	0 (0.0%)		
2	0 (0.0%)	0 (0.0%)		
3	1 (9.1%)	0 (0.0%)		
Acute Kidney Injury	2 (18.8%)	0 (0.0%)		0.096
Inotrope score				
Post bypass	8.60 (17.27)	3.98 (9.33)	4.62 (-7.03, 16.28)	0.419
24 hours post-operatively	13.84 (26.74)	5.02 (6.76)	8.82 (-7.12, 24.77)	0.263
48 hours post-operatively	13.20 (18.75)	1.13 (2.84)	12.07 (1.17, 22.97)	0.032
72 hours post-operatively	3.82 (8.04)	0.00 (0.00)	3.82 (-0.79, 8.42)	0.100
Total	39.78 (64.53)	10.12 (15.72)	29.66 (-8.73, 68.05)	0.123
New onset AF	4 36.4%)	1 (7.1%)		0.070
Length of ICU stay (days)	3.0 (2.0-4.5)**	1.0 (1.0-2.0)**		0.096***
Length of hospital stay (days)	9.0 (7.0-14.5)**	6.5 (6.0-8.0)**		0.118***
Clinical outcomes at six weeks				
Death	0 (0.0%)	0 (0.0%)		1.000
Myocardial infarction	0 (0.0%)	0 (0.0%)		1.000
Stroke	0 (0.0%)	0 (0.0%)		1.000
Revascularization	0 (0.0%)	0 (0.0%)		1.000

Table 6.20. Summary of study endpoints in non-diabetic patients undergoing CABG surgery with cardioplegia

Endpoint	Control (n=18) (mean [SD])	RIPC (n=23) (mean [SD])	Difference (95% CI)	P value
CK (*µg/L*)				
Total AUC	30221.65 (13177.39)	35801.29 (25015.85)	-5579.64 (-19217.85, 8058.57)	0.412
Creatinine (mg/ml)				
Pre-operatively	88.0 (19.8)	81.3 (22.1)	6.7 (-4.6, 17.9)	0.243
24 hours post-operatively	84.1 (25.4)	82.0 (19.8)	2.1 (-10.0, 14.3)	0.729
48 hours post-operatively	93.1 (39.5)	84.9 (25.5)	8.2 (-9.5, 25.9)	0.357
72 hours post-operatively	89.2 (43.1)	86.9 (27.2)	2.3 (-16.9, 21.4)	0.814
Urine Output (ml)				
24 hours post-operatively	2029.7 (556.0)	2223.5 (509.6)	-193.8 (-500.5, 112.9)	0.210
48 hours post-operatively	2324.9 (1097.6)	2291.6 (807.6)	33.3 (-545.8, 612.5)	0.908
72 hours post-operatively	2039.6 (836.3)	2264.8 (811.8)	-225.2 (-852.4, 401.9)	0.468
Total	6225.8 (2229.0)	6465.4 (1509.9)	-239.7 (-1634.0, 1154.6)	0.727
AKI score				
0	7 (70.0%)	8 (88.9%)		0.528
1	1 (10.0%)	0 (0.0%)		
2	1 (10.0%)	1(11.1%)		
3	1 (10.0%)	0 (0.0%)		
Acute Kidney Injury	10 (38.5%)	9 (31.0%)		0.511
Inotrope score				
Post bypass	5.44 (9.64)	78.36 (19.90)	-2.92 (-11.71, 5.84)	0.508
24 hours post-operatively	8.00 (14.50)	10.24 (19.78)	-2.24 (-12.04, 7.57)	0.648
48 hours post-operatively	4.46 (10.29)	6.39 (17.42)	-1.93 (-10.22, 6.36)	0.642
72 hours post-operatively	1.09 (2.15)	0.76 (2.39)	0.33 (-0.96, 1.62)	0.610
Total	19.23 (30.74)	25.75 (51.49)	-6.51 (-31.07, 18.04)	0.596
New onset AF	6 (22.2%)	4 (13.8%)		0.411
Length of ICU stay (days)	2.0 (1.0-3.0)**	2.0 (2.0-3.0)**		0.321***
Length of hospital stay (days)	8.0 (6.5-11.0)**	7.0 (6.0–10.0)**		0.903***
Clinical outcomes at six weeks				
Death	0 (0.0%)	0 (0.0%)		1.000
Myocardial infarction	0 (0.0%)	0 (0.0%)		1.000
Stroke	0 (0.0%)	0 (0.0%)		1.000
Revascularization	0 (0.0%)	0 (0.0%)		1.000

*List of abbreviations

RIPC=Remote ischemic preconditioning; CK=Creatinine Kinase; AKI=Acute Kidney Injury; AF=atrial fibrillation; ICU=Intensive Care Unit; AUC=Area-under-the-curve. **Results shown as median (inter-quartile range); *** P-value for Mann-Whitney-Wilcoxon test

6.7. Discussion

In the most recent subgroup analyses, we have demonstrated that simultaneous multi-limb IR enhanced cardioprotection in non-diabetic patients undergoing unselected cardiac surgery as well as those having any cardiac surgery and receiving cardioplegia intra-operatively. Crucially we have also showed that our enhanced preconditioning stimulus decreased PMI and reduced the rate of new onset post-operative AF in diabetic patients undergoing CABG surgery although similar subjects receiving cardioplegia were not significantly protected.

Only a few studies investigating the effects of RIPC on PMI included diabetic patients: in literature we found 7 RCTs excluding subjects with DM (286, 291, 293, 297-299, 302), 8 RCTs including both diabetic and non-diabetic patients (282, 285, 294-296, 300, 303, 305) and 5 RCTs not clearly documenting the inclusion or exclusion of diabetic patients (284, 301, 304, 306, 445) **(Table 6.21)**. Importantly, even when diabetic subjects were enrolled, those taking glibenclamide were excluded due to its interference with RIPC-induced cardioprotection (549).

Our group conducted the first major clinical study (291) including 45 non-diabetic patients undergoing CABG with or without valve surgery with cold cardioplegia: subjects randomised to standard RIPC sustained a significantly reduced PMI, similarly to our subgroup analysis comprising a larger cohort of 107 non-diabetic patients having unselected cardiac surgery with cardioplegia.

Rahman et al (286) failed to demonstrate myocardial protection in non-diabetic patients undergoing CABG surgery with cardioplegia: similarly, when we analised data from non-diabetic patients having CABG surgery only with cardioplegia, we only obtained a non-significant 13% reduction of 72 hour AUC, although in this retrospective study our cohort size was remarkably reduced to 43 patients only.

Table 6.21. Major clinical studies investigating the effects of RIPC in CABG surgery

Group	Number of patients and RIPC Stimulus	Patient group and surgery setting	Cardioprotection achieved
Diabetic patients excluded			
Venugopal (291) (2009)	45 Upper-limb ischaemia (3 cycles of 5 min)	Elective CABG±Valve Surgery	Yes
Thielmann (293) (2010)	53 Upper-limb ischaemia (3 cycles of 5 min)	Elective CABG	Yes
Rahman (286) (2010)	162 Upper-limb ischaemia (3 cycles of 5 min)	Elective CABG	No
Li (302) 2010	81 Lower limb ischemia (3 x 4 min) before (RIPC) or after (RIPerC) aortic cross-clamping	Elective valve replacement	Only in RIPerC group
Lomirotov (297) 2012	80 Upper-limb ischemia (3 cycles of 5 min)	Elective CABG	No
Kottenberg (298) 2012	72 Upper-limb ischemia (3 cycles of 5 min)	Elective CABG	Only with RIPC given with isoflurane and not propofol
Kim (439) 2012	54 Three-10 min cycles of leg ischemia prior to and after bypass	Elective complex valve surgery	Not assessed
Lucchinetti (299) 2012	55 Lower limb ischaemia (4 cycles of 5 min)	Elective CABG	No
Diabetic patients included			
Hausenloy (282) (2007)	57 Upper-limb ischaemia (3 cycles of 5 min)	Elective CABG	Yes
Wagner (295) 2010	101 Upper limb ischaemia (3 cycles of 5 min)	Elective CABG	↓TnI at 8 hours only Tramadol group had↑ TnI at 8, 16 and 24 hours.
Choi (303) 2011	76 Lower limb ischemia (3 x 10 min)	Complex valve surgery	Significant CK-MB reduction at 24 hours only
Karuppasamy (294) 2011	54 Upper-limb ischaemia (3 cycles of 5 min)	Elective CABG surgery	No
Hong (285) 2012	70 Lower-limb ischaemia (4 cycles of 5 min)	Elective off-pump CABG	Yes
Young (296) 2012	96 Upper-limb ischemia (3 x 5 min)	High risk cardiac surgery	No
Thielmann (300) 2013	329 Upper-limb ischaemia (3 cycles of 5 min)	Elective CABG	Yes

Not clearly documented if diabetic patients included			
Li (301) 2001	40 Aortic cross-clamping (two-3 minutes cycles of ischaemia and 2 minutes of reperfusion)	MVR, AVR, DVR	Improved pulmonary function and decreased inflammatory response
Wu (306) 2011	40 Upper-limb ischaemia (3 cycles of 5 min) with or without upper leg ischaemia (2x10 min cycles)	MVR	Reduced TnI release in combined upper arm and upper thigh IR group only
Xie (304) 2011	73 Upper-limb ischemia (3 cycles of 5 min)	Elective valve replacement	Yes
Hong (284) 2010	130 Upper-limb ischaemia (4 cycles of 5 min)	Elective off-pump CABG	No
Ali (292) 2010	100 Upper-limb ischaemia (3 cycles of 5 min)	Elective CABG	Yes

DVR=aortic and mitral valve replacement; MVR=mitral valve replacement; LIPC=limb ischaemic preconditioning; RIPC=remote ischemic preconditioning; CABG=coronary artery bypass graft; cTnI=cardiac troponin I; cTnT=cardiac troponin T CK=creatine kinase; AUC=area-under-the-curve; RIPost=remote ischaemic postconditioning; CKD=chronic kidney disease; DM=diabetes mellitus; LVEF=left ventricular ejection fraction; CPB=cardiopulmonary bypass; BNP=brain natriuretic peptide; CRP=C-reactive protein; MV=mitral valve.

In another study including 55 non-diabetic patients undergoing CABG surgery with cardioplegia, where a potent preconditioning stimulus of four-5 minutes cycles of lower limb IR (299), again no cardiac or neurological protection or clinical outcomes improvement were showed. In three other major RCTs excluding diabetic patients (293, 297, 298), discrepant results on cardioprotection outcomes were obtained and only the study from Thielmann's group (293) showed significant PMI reduction. However it is crucial to notice that in these studies, crystalloid cardioplegia was used, in contrast to blood cardioplegia utilised at the centre where we conducted our trial.

In summary, proof-of-concept studies on diabetic or non-diabetic patients have reported discrepant results: whilst experimental studies have suggested the possibility

of inducing cardioprotection in the diabetic heart albeit with an increased preconditioning stimulus, clinical studies have so far not entirely confirmed these findings, although no trial has yet been conducted with the inclusion of diabetic subjects only. Intriguingly, in a retrospective analysis of patients undergoing CABG surgery (550), standard RIPC was associated with:

- significant reduction of cTnI AUC by 41% in non-diabetic patients;
- significant increase of 56% AUC in sulphonylurea-treated diabetics versus non-diabetics;
- no significant change of PMI in:
  - ✓ all diabetics patients, regardless hypoglycaemic treatment administered;
  - ✓ non-sulphonylurea-treated diabetics (i.e. those treated with metformin or insulin);
  - ✓ non-sulphonylurea-treated diabetics compared to diabetics treated with other drugs;

However, this was a retrospective analysis and certainly not powered for the designated endpoints and no control group of non-diabetics receiving sulphonylurea therapy was included, although of course for ethical reasons. Moreover, the study could not clarify the reasons for the discrepant results between sulphonylurea-treated diabetics and non-diabetics, and in particular whether these could be secondary to molecular effects of sulphonylurea medications, or to diabetes in itself or a combination of the two or even arterial blood glucose concentrations peri-operatively. Crucially, 40% of the diabetics in this study received the sulphonylurea glibenclamide, which has been demsontrated to interefere with IPC induced cardioprotection by blocking K_{ATP} channels in dogs (172, 551), pigs (552-554), and humans (555, 556). In

our study, we excluded diabetic patients on glibenclamide for this reason, and only gliclazide was used amongst sulphonylureas, which in contrast with the non-selective action of glibenclamide on pancreatic β-cells, vascular myocytes and cardiomyocytes, is a highly pancreatic selective segretagogue and thereby does not abolish IPC effects.

In addition, when we then went on to analyse any potential difference in PMI between control diabetics and control non-diabetics, we found no statistically significant difference in total hsTnT release in any of the subgroup analyses we mentioned in the current chapter.

Table 6.22. Comparison of peri-operative myocardial injury in control patients

Patients	DM (mean (sd))	Non-DM (mean (sd))	Difference (95% CI)	P value
All diabetics (n=24) versus all non-diabetics (n=62)	35.99 (21.86)	36.43 (25.67)	-0.44 (-12.24, 11.37)	0.942
Cardioplegia diabetics (n=18) versus Cardioplegia non-diabetics (n=52)	34.88 (20.58)	37.85 (27.43)	-2.97 (-17.09, 11.16)	0.676
CABG diabetics (n=17) versus CABG non-diabetics (n=36)	31.73 (18.63)	30.29 (19.34)	1.44 (-9.86, 12.73)	0.800
CABG-cardioplegia diabetics (n=11) versus CABG-cardioplegia non-diabetics (n=26)	27.59 (11.46)	30.78 (21.81)	-3.19 (-17.37, 10.99)	0.650

DM=diabetes mellitus; sd=standard deviation; CI=confidence interval; CABG=coronary artery bypass graft

This is in contrast with the literature on animal models, showing that diabetic myocardium is more resistant to IRI, and potentially reflects crucial differences between animal and human models, including (457):

- ✓ the single specific disease of animal models versus the presence of multiple co-morbidities in the vast majority of patients with CAD potentially able to impact on RIPC-induced cardioprotection;
- ✓ concomitant multiple drug therapy for humans which can again potentially interfere with preconditioning in contrast with animal models;
- ✓ the frequent presence of LV hypertrophy/impairment in these patients with subsequent interaction with IRI;
- ✓ the relatively advanced age of patients, which can potentially reduce the RIPC effects, versus young animal models used in the vast majority of experimental studies;
- ✓ the use of type 1 DM models in the majority of preclinical studies, in contrast with human models, where 90% of DM cases are Type 2 DM.

Crucially, a parallel unpublished study conducted at our Institute included diabetic and non-diabetic patients undergoing elective CABG surgery randomised to receive either control or RIPC comprising three-5 min cycles of upper arm IR or RIPC consisting of two-5 min cycles of simultaneous upper and lower limb. Atrial trabeculae were isolated from the right atrial appendage and subjected to 90 minutes of simulated ischaemia and 120 minutes of simulated reperfusion, at the end of which the recovery of baseline contractile function was determined. Atrial trabeculae harvested from 13 diabetic and 20 non-diabetic control patients were demonstrated to recover 24.5±2.4% and 29.3±1.3% of baseline contractile function, respectively. Treatment with standard

RIPC increased the recovery of baseline contractile function in both non-diabetic (50.38±1.946%) and diabetic patients (41.55±1.946%), however, our simultaneous multi-limb preconditioning stimulus resulted in a greater recovery of baseline contractile function in both non-diabetic (59.25±1.942%) and diabetic patients (50.74±2.131%). As a positive control direct hypoxic preconditioning (HPC) of atrial trabeculae also improved the recovery of baseline contractile function (56.4±1.8% with HPC vs 27.5±1.7% in control; n=10 patients; $p<0.0001$). Therefore this study was the first to demonstrate that *in vivo* RIPC can protect *ex vivo* atrial trabeculae against simulated IRI and confirmed that RIPC is able to produce a graded cardioprotective response.

In summary, we demonstrated that our enhanced preconditioning stimulus significantly reduced total hsTnT release from 31.73±18.63 µg/L to 19.63±9.19 µg/L [12.09; CI 1.83, 22.35; $p=0.022$] and the incidence of new onset post-operative AF from 6 to 1 ($p=0.023$) in diabetics patients undergoing elective CABG surgery with either cardioplegia or ICCF but not in those having cardioplegia only. We therefore showed for the first time that an enhanced RIPC stimulus is potentially able to overcome the higher preconditioning threshold required in order to achieve significant cardioprotection. In addition, we also demonstrated that, in contrast with animal models, human diabetic myocardium is not more resistant to IRI than non-diabetic hearts. However, again our findings derive from a relatively small cohort of patients and will need to be confirmed in a larger clinical trial: in this regards, our ERICCA will again provide the final conclusion as to whether an enhanced preconditioning stimulus will improve PMI and short and long-term clinical outcomes in these higher risk patients undergoing elective CABG surgery with or without valve surgery (290).

CHAPTER 7

7. Effects of RIPC on clinical outcomes in high-risk patients undergoing CABG surgery with or without valve surgery (ERICCA Trial)

7.1. Introduction and Rationale

CABG surgery is increasingly becoming the treatment strategy of choice in patients with multi-vessel CAD, particularly involving LMS and/or proximal LAD, with an age greater than 65 years and with known DM (13, 14). The risk profile of patients undergoing CABG surgery continues to change due to factors such as (a) the aging population (the proportion of patients over 75 years old has increased by more than 4.5-fold over the last decade with a 5-year mortality in this age group of 35%); (b) the increasing prevalence of diabetes (the proportion of diabetic patients has risen from 15% to 22%, with an operative mortality of 2.6%) resulting in an increase in the number of higher-risk patients (defined as an additive EuroSCORE greater than or equal to 5) being operated on and a corresponding increase in overall operative risk to 5-6% (33, 34). These higher-risk patients are at a greater risk of requiring inotropic support post-surgery and sustaining PMI, AKI (557) and stroke (1-3%) (558), resulting in worse clinical outcomes. Crucially, PMI has been associated with worse clinical outcomes following surgery (77-82). Moreover, the incidence of AKI post-cardiac surgery can be as high as 34% with up to 2% of patients requiring dialysis (364, 559,

560), which increases the risk of death 7.9 times (365). Furthermore, it has been reported that changes greater than 0.5 mg/dl (44 mmol/L) in creatinine after cardiac surgery also contribute to a significant increase in mortality at 30 days post-surgery (463). Clearly, new treatment strategies are required to protect the heart, the brain and the kidney during higher-risk CABG with or without valve surgery, in order to that improve clinical short and long term clinical outcomes in this patient group. In this regard, despite often discordant outcomes from the considerable number of relatively small proof-of-concept clinical studies so far published, RIPC has been demonstrated to be a simple, non-invasive, risk and cost-free intervention able to enhance the innate mechanism of cardioprotection and thereby to reduce myocardial damage in patients undergoing cardiac surgery (282, 284, 285, 291, 293-299, 302-306, 445, 469). However, little data are available on the clinical significance of the impact of RIPC on PMI **(Table 7.1)**: at the time of our current study initiation no RCT had determined whether long-term morbidity and mortality are improved in preconditioned patients receiving cardiac surgery. We therefore conducted a multi-centre, double-blinded randomised-control clinical trial in order to establish the effects of RIPC on clinical outcomes on high-risk patients undergoing CABG surgery with or without valve surgery (ERICCA trial) (290).

Table 7.1. Summary of major clinical studies investigating clinical outcomes following discharge post-adult cardiac surgery

Author	Type of surgery	Clinical Outcomes	Mean EuroSCORE
Li (301) 2001	MVR, AVR, DVR	No difference in mortality rate at 30 days post-surgery	No mean EuroSCORE reported
Hausenloy (282) (2007)	Elective CABG	No clinical outcome	RIPC 3.2 (2.6) Control 3.3 (2.4)
Venugopal (291) (2009)	Elective CABG ± Valve Surgery	No clinical outcome	RIPC 2.1 (1.9) Control 2.6 (2.1)
Ali (292) 2010	Elective CABG	No clinical outcome (abstract only)	No mean EuroSCORE reported (abstract only)
Thielmann (293) (2010)	Elective CABG	No difference in MACCE at 30 days post-surgery	Additive EuroSCORE RIPC 3.5 (2.0) Control 2.8 (2.2) Logistic EuroSCORE RIPC 3.0 (1.8) Control 2.4 (1.7) STS Score (%) RIPC 0.76 (0.49) Control 0.86 (0.73)
Rahman (286) (2010)	Elective CABG	No clinical outcome	RIPC 3 (IQR: 2, 4.5) Control 3 (IQR: 2, 5)
Li (302) 2010	Elective valve replacement	No clinical outcome	No mean EuroSCORE reported
Karuppasamy (294) 2011	Elective CABG surgery	No clinical outcome	RIPC 4.26 (2.03) Control 3.78 (2.15)
Hong (284) 2010	Elective off-pump CABG	No clinical outcome (abstract only)	No mean EuroSCORE reported (abstract only)
Wagner (295) 2010	Elective CABG	No clinical outcome	RIPC 5 (IQR: 1-6) Control 5 (IQR: 2-8) Tramadol 4 (IQR: 1-10)
Choi (303) 2011	Complex valve surgery	No clinical outcome	RIPC 3.1 (1.4) Control 3.5 (2.4)
Wu (306) 2011	MV surgery	No clinical outcome	No mean EuroSCORE reported
Xie (304) 2011	Elective valve replacement	Improved NYHA status and LVEF at 3 months post-surgery	No mean EuroSCORE reported
Young (296) 2012	High risk cardiac surgery	No clinical outcome	RIPC 7.1 (6.1) Control 6.6 (6.1)
Lomirotov (297) 2012	Elective CABG	No difference in mortality rate at 30 days post-surgery	RIPC 2.2 (0.6) Control 2.5 (0.8)
Kottenberg (298) 2012	Elective CABG	No clinical outcome	No mean EuroSCORE reported
Hong (285) 2012	Elective off-pump CABG	No clinical outcome	Logistic EuroSCORE RIPC 2.1 (IQR: 1.5-3.1) Control 1.8 (1.3-3.5)
Kim (305) 2012	Complex valve surgery	No clinical outcome	No mean EuroSCORE reported
Lucchinetti (299) 2012	Elective CABG	Higher incidence in RIPC group for peri-operative composite end-point of new arrhythmias and MI but no difference at 6 months	No mean EuroSCORE reported

Thielmann (300) 2013	Elective CABG	Reduced all-cause mortality rate and MACCE at 1.5 years (mainly driven by reduced MI rate)	Additive EuroSCORE RIPC 4.7 (1.9) Control 4.9 (2.0) Logistic EuroSCORE RIPC 4.1 (2.8) Control 4.6 (4.0) EuroSCORE II(%) RIPC 1.2 (0.5) Control 1.2 (0.5)

RIPC=remote ischaemic preconditioning, MACCE=major cardiovascular and cerebrovascular events; CABG=coronary artery bypass graft; MVR=mitral valve replacement; AVR=aortic valve replacement; DVR=double valve replacement; IQR=interquartile range; NYHA=New York health association

7.2. Aims and Objectives

7.2.1. Hypothesis

We hypothesised that RIPC induced by brief arm IR improves clinical outcomes at one year in higher-risk adult patients undergoing CABG with or without valve surgery compared to control (PICO=Population, Intervention, Comparator, Outcome).

7.2.2. Overall Aim

To determine whether RIPC improves clinical outcomes at one year in high-risk patients undergoing CABG surgery with or without valve surgery.

7.2.3. Objectives

7.2.3.1. Primary research objective

To determine whether RIPC improves one year clinical outcomes in patients undergoing CABG with or without valve surgery.

7.2.3.2. Secondary research objectives

To determine whether, in patients undergoing CABG with or without valve surgery, RIPC:

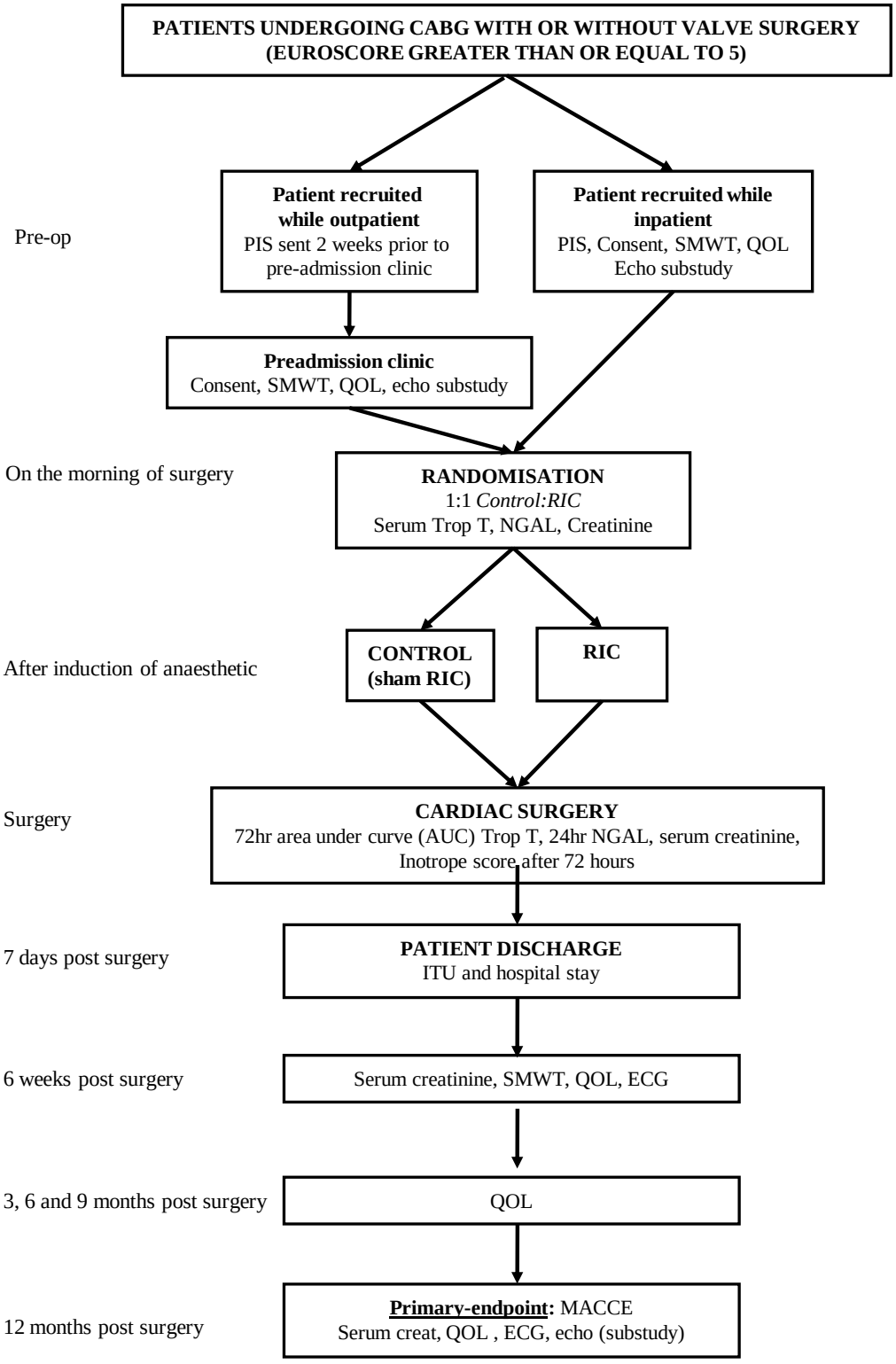
a. improves 30-day clinical outcomes;
b. has an effect on all-cause death;
c. reduces PMI;
d. reduces AKI and preserves renal function;
e. improves patient morbidity, assessed by parameters such as:
    i. ITU stay,
    ii. inotrope score,
    iii. six minute walk test,
    iv. quality of life assessment;
f. improves LVEF measured by echocardiography in a substudy of patients recruited via selected hospitals.

7.3. Methods

7.3.1. Overview

We conducted a multi-centre double-blinded randomised control clinical trial to investigate the effects of RIPC on clinical outcomes in high-risk patients undergoing CABG with or without valve surgery. A schematic overview of study design is given in **Fig. 7.1.**

Fig. 7.1. Study Synopsis



CABG=coronary artery bypass surgery; PIS=patient information sheet; SMWT=six minute walk test; QOL=quality of life questionnaire; RIC=remote ischaemic conditioning; AUC=area under the curve; NGAL=neutrophil gelatinase associated lipocalin; ITU= intensive therapy unit; ECG=electrocardiogram; MACCE=major adverse cardiovascular and cerebrovascular events.

7.3.2. Ethical approval, informed consent and ethical considerations

The study conformed to the spirit and the letter of the declaration of Helsinki, and in accordance with the UCL Good Clinical Practice Guidelines. Patients gave their informed consent to participate in the study and could decide to withdraw from the study at any time without prejudice to their future care.

Patients' recruitment and consent occurred through two different pathways, the preadmission clinic, approximately 2 weeks prior to surgery, where eligible patients who had already received the patient information sheet (PIS) were given further full explanation of study details and potential implications, and the cardiac ward, where subjects awaiting surgery at the recruiting hospital were identified, given the PIS and further approached after 24 hours for consent, thereby after being given sufficient time to be able to give an informed consent.

7.3.2.1. Ethical committee review. East London 3 Research Ethics Committee reviewed and approved the trial (10/H0701/111). Copies of the approval letters were filed in the study files at each centre. Previous Ethical Approval was already in place to investigate RIPC in the setting of CABG with or without valve surgery (REC Ref: 06/20502/83).

7.3.2.2. Data handling and record keeping. Electronic data will be returned to the London School of Hygiene and Tropical Medicine (LSHTM) and kept for 15 years following completion of the study. The use of the data from the study will be controlled by the chief investigator and the Clinical Trials Unit at the LSHTM. A signed hard-copy of the RIPC intervention sheet WAS kept at each centre and copied to the Clinical Trials Unit at the LSHTM.

7.3.3. Study Design

7.3.3.1. Primary research objective

A multi-centre double-blinded randomised controlled trial to investigate whether RIPC improves clinical outcomes at one year in high-risk patients undergoing CABG with or without valve surgery.

7.3.3.2. Number of centres

The trial, co-ordinated by the Clinical Trials Unit, LSHTM (London), recruited patients were from the following 30 centres:

- UCLH Heart Hospital
- King's College London Hospital
- Papworth Hospital
- Hammersmith Hospital
- St Thomas' Hospital
- Essex Cardiothoracic Centre
- Royal Sussex County Hospital
- Royal Brompton Hospital
- Harefield Hospital
- Derriford Hospital
- Manchester Royal Infirmary
- Swansea Morriston Hospital
- Edinburgh Royal Infirmary
- St Barts' Hospital
- London Chest Hospital
- St George's Hospital
- North Staffordshire University Hospital
- University Hospital, Galway
- Southampton General Hospital
- Cardiff University Hospital
- Golden Jubilee Hospital
- Leeds General Infirmary
- University Hospital, Coventry
- Blackpool Victoria Hospital
- Trent Cardiac Centre
- Northern General Hospital
- Castle Hill Hospital
- Glenfield Hospital
- Wythenshawe Hospital
- Wolverhampton Hospital

7.3.3.3. Patient Recruitment

Adult patients were screened against inclusion and exclusion criteria and recruited if considered eligible to the study.

7.3.3.3.1. Inclusion criteria

a. Patients aged 18 years and above.
b. Patients undergoing CABG with or without valve surgery using blood cardioplegia.
c. Patients with an additive EuroSCORE greater than or equal to 5. This was calculated with the Microsoft excel EuroSCORE calculator (http://www.euroscore.org/calculators) (47) and is an accepted criterion for defining higher-risk patients.

7.3.3.3.2. Exclusion criteria

a. Cardiogenic shock or cardiac arrest on current admission (see chapter 3).
b. Pregnancy.
c. Significant PAD affecting the upper limbs.
d. Patients with significant hepatic dysfunction (INR>2)
e. Patients with significant pulmonary disease (FEV1<40% predicted).
f. Patients with known renal failure with an eGFR<30 mL/min/1.73 m^2.
g. Patients on glibenclamide or nicorandil, as these medications may interfere with RIPC (171, 441)
h. Patients recruited into another study, which might have impacted on the ERICCA study.

7.3.3.3.4. Randomisation

The randomisation procedure, co-ordinated centrally by the LSHTM, was carried out as close as possible to the time of surgery, via a secure web site and stratified by centre using random permuted blocks. This was only accessed by the research nurse responsible for performing either the RIPC or control protocol, who was the only

person in each centre aware of the treatment allocation for the patient and was not involved in the data collection other than those relating to the actual randomisation procedure.

7.3.3.3.5. Treatment allocation and Method of blinding

Treatment allocation was only known by one research nurse at each centre. Patients, cardiac surgeons, the research nurse collecting the data, and the assessor of clinical outcomes were blinded to the treatment allocation. A research nurse at each study site remained blinded to the allocation of patients to either real or sham RIPC. The preconditioning procedure was performed by an investigator not involved in sample collection or data analysis.

7.3.3.3.6. Withdrawal from study

Patients could decide to withdraw from the study at any time without prejudice to their future care, although this was uncommon, because of the non-invasive nature of the intervention and the follow-up, which was integrated within routine clinical care wherever possible. We allowed in our sample size calculation for a drop-out rate of up to 5% (from the SYNTAX trial(561)) although it was expected to be lower than this.

Patients were encouraged to allow data and samples collected before withdrawal to be used in the analyses. However, if consent to use data/samples was also withdrawn, then these were discarded. Patients withdrawing from the study were to be continued to be followed-up by their local team. There should be no need for further follow-up from the research team.

7.3.3.4. Intervention: RIPC and sham treatment protocols

RIPC was applied after anaesthesia induction and consisted of inflation of a standard BP cuff applied to the upper arm to 200mmHg for 5 minutes, then deflated for 5 minutes, a cycle which was repeated 4 times in total. For patients with SBP>185mmHg, the cuff was inflated to at least 15mmHg above the patient's SBP. The sham RIC protocol, applied after anaesthesia induction, was delivered using a standard BP cuff as follows: the air valve on the BP cuff was first opened such that the cuff was not inflated on squeezing the attached bulb. The bulb was then squeezed for a duration of 15 seconds to give the impression that the cuff was being inflated. After 5 minutes the air valve was closed to give the impression that the cuff was being deflated. After 5 minutes, the air valve was opened again and the bulb squeezed as before. This cycle was repeated 4 times in total. These interventions were not to prolong the anaesthetic time or delay the onset of surgery.

7.3.3.5. and 7.3.3.6. Anaesthetic procedure and Surgical Procedure

These have already been discussed in chapter 3.

7.3.3.7. Study Endpoints: rationale and assessment

7.3.3.7.1. Study Primary Endpoint.

The study primary endpoint is the MACCE rate at 1 year post-surgery, comprising death, MI, revascularisation and death (these have already been defined in chapter 3.

7.3.3.7.2. Study Secondary Endpoint.

7.3.3.7.2.1. 30 day MACCE

7.3.3.7.2.2. All cause death

7.3.3.7.2.3. PMI

This was assessed by measuring serum hsTnT pre-operatively and at 6, 12, 24, 48, 72 hours post-surgery: several studies (77-82) have demonstrated that following cardiac surgery, PMI, indicated by cardiac biomarkers rise in the post-operative period, is associated with worse short and long-term clinical outcomes. Assay details and AUC calculation have already been discussed in chapter 3. Each blood sample was labelled, centrifuged, divided into two samples, aliquoted, frozen (at -20ºC) and stored locally. Every quarterly period throughout the 2-year recruitment period batches of samples were couriered from the recruitment centres to The Doctors' Laboratories in London for analysis.

7.3.3.7.2.4. AKI

The rationale for the evaluation of AKI has already been elucidated in chapter 3.

7.3.3.7.2.5. Creatinine

Creatinine will be measured pre-operatively and daily for the first three post-operative days, at 6 weeks and one-year post-CABG with or without valve surgery.

7.3.3.7.2.6. Neutrophil Gelatinase Associated Lipocalin (NGAL)

Plasma NGAL is a new early marker of AK, with levels rising rapidly following renal damage, and has been used to evaluate renal injury in the context of cardiac surgery (562, 563). NGAL was measured at 4 time-points: pre-operatively, 6, 12 and 24 hours post-coming off cardiac bypass, from which a 24 hours AUC was calculated. The NGAL Rapid ELISA Kit measures human NGAL in plasma/serum, with a positive predictive value for acute renal failure is over 90% (562, 563). Each NGAL blood sample was labelled, centrifuged, then plasma was divided into two samples, frozen (within 4 hours of collection) and stored locally at -20 ºC. Every quarter throughout the 2 year recruitment period batches of samples were couriered to a single laboratory for analysis (Caltag Medsystems, Buckingham, UK).

7.3.3.7.2.7. Inotrope requirement

The rationale for this study end-point has been discussed in chapter 2. Data on inotrope use were collected daily from the medical drug chart on the ICU and the inotrope score will be calculated using the cited formula from Ko et al (443) at 0 (time when coming off bypass), 24, 48 and 72 hours after the surgery. We expect RIPC to impact on this outcome measure by reducing PMI and therefore preserving LV systolic function.

7.3.3.7.2.8. and 7.3.3.7.2.9. Duration of ICU and hospital stay

These have already been discussed in chapter 3.

7.2.3.10.2.10. The Six minute walk test (6MWT)

The 6MWT was used to evaluate the functional status of patients undergoing CABG with or without valve surgery (564). Shortly after CABG with or without valve surgery, functional capacity is significantly reduced, but rapidly improves after cardiac rehabilitation. This has been found to be independent of age, sex, co-morbidities and baseline functional capacity (564). The 6MWT was performed at baseline, 6 weeks, and one year. Patients were instructed to walk as far as possible along a straight, flat hospital corridor in 6 minutes.

7.3.3.10.2.11. Quality of life

A retrospective analysis on 1180 patients undergoing CABG surgery between 1994 and 1996 showed that of 621 patients assessed for quality of life at 10 years, 85% had a quality of life within a 95% CI of the score found in the general population with similar age and that 14.7% of patients reported poor quality of life (565). Significant predictors of poor long-term quality of life were current smoking, CCS grade III or IV, redo-operation, female sex, DM, PAD, more than 2-day stay on ICU, and COPD. Interestingly 25% of patients with poor outcome of Health-Related Quality of Life (HRQOL) questionnaire had grade IV angina. The HRQOL questionnaire (www.euroqol.org) was used to assess patient quality of life post-CABG with or without valve surgery, at baseline, 6 weeks, 3, 6 and 9 months and one year. For details on HRQOL, see Appendix.

7.3.3.10.2.12. ECHO Substudy

LVEF post-CABG with or without valve surgery is a strong determinant of clinical outcome (1): a subgroup of 140 patients at a selection of centres had a transthoracic

echocardiogram (TTE) performed at baseline to assess LVEF (by bi-planar Simpson's technique and 3D techniques) either in the surgical pre-admission clinic or as an in-patient prior to surgery and was to be repeated at one year.

We intended to assess LV dimensions and LV volumes, LVEF, global peak systolic strain in radial and longitudinal axis, and mitral annular plane systolic excursion, RVEF by fractional area change and tricuspid annular plane systolic excursion through a standardised protocol:

1. Acquisition includes 3 short-axis views at MV, papillary muscle and apical levels, and 4 apical views LV 4-chamber, RV 4-chamber, LV 2-chamber and LV 3-chamber views.
2. When 3D imaging was available, 2 full volume acquisitions were acquired during breath holding with 4 beats averaging.
3. Detection of severe valvular disease and other abnormalities were reported.

Only recent echo machines from GE and Philips were used for the study. The Echo core laboratory at the Heart Hospital was in charge of analysis, interpretation, quality control, observer and centre variability, and echo database.

7.3.3.2.13. Genetic and Biomarker analysis

We also intend to perform genetic and biomarker analysis in order to evaluate expressions of genes and protein synthesis implicated in the mechanistic pathways of RIPC. At the time of writing of this thesis, specific targets of this analysis had not yet been determined, however we feel that the collection of such a considerable amount of data gives us the opportunity to have a very valuable resource available for further understanding mechanisms involved in RIPC.

7.3.3.8. Statistical considerations and sample size determination

7.3.3.8.1. Primary clinical endpoints

We originally planned to recruit 1610 patients through 30 surgical centres. In the SYNTAX study (14) the MACCE rate was 12.4% of patients at 12 months following CABG surgery. However, the patients recruited into the SYNTAX study were low-risk with a mean EuroSCORE of 3.8±2.7, whereas the patients we recruited in our study had a EuroSCORE greater than or equal to 5. In another study comprising higher-risk patients with LMS disease, the MACCE rate at one year was estimated to be 25% (566). Therefore, for our higher-risk CABG with or without valve surgery patients we estimated a MACCE rate of 20% at one year and in order to detect a 27% relative reduction in this primary endpoint in the RIPC-treated group (from 20.0% to 14.6%), with a power of 80% and a significance level of 5%, a sample size of 770 patients were required for each trial arm (1540 in total). A trial of this size would be able to detect an observed relative reduction of 20% (i.e. a risk ratio of 0.8) as statistically significant based on an event rate in the control arm of 20%. To allow for dropouts (4.5% in the SYNTAX study), we planned to recruit 1610 patients in total (805 patients each arm).

Prior to the start of our study, we intended to recruit over a 24-month period as we expected an enrolment of approximately 3 high-risk patients per month at each of the recruiting centres. At least 80% of patients undergoing CABG surgery in our recruiting centers had an additive EuroSCORE greater than or equal to 5. Each centre operates on about 5-6 high-risk surgical patients per week, meaning that we needed to recruit at least 25% of the eligible patients.

Survival analyses techniques will be used for MACCE and other clinical endpoints. Hazard ratios and confidence intervals will be calculated using Cox

proportional hazards modelling and Kaplan-Meier curves will be produced. The assumptions underlying the Cox model will be assessed. In addition risk differences at one-year together with 95% confidence intervals will be calculated. Differences in means (continuous variables) together with 95% confidence intervals will be calculated using linear regression models and analysis of covariance techniques where appropriate. Analysis will be by intention to treat using all available data. The subgroups will be analysed using interaction tests. A Data Management Committee (DMC) will be convened to periodically review data. This will be the only group, along with the statistician producing the reports for the DMC, who will see interim analyses by treatment.

7.3.3.8.2. Secondary clinical endpoints

7.3.3.8.2.1. PMI

Our group demonstrated that RIPC reduced PMI by 43% in patients undergoing CABG surgery from 36.1 µg/L.72hrs to 20.6 µg/L.72hrs (282). The mean difference was 15.5 ng/ml with a pooled SD of 17.8 µg/L. To demonstrate such a difference as being statistically significant at the 5% level, with 90% power, 28 patients per group were required per group.

7.3.3.8.2.2. Inotrope score

Cheung et al (281) demonstrated that RIPC reduced the inotrope score 3 hours post-operatively by 29% in children undergoing corrective cardiac surgery from 11.4µg/kg/min to 8.1µg/kg/min. The mean difference was 3.3µg/kg/min with a SD of

4.1µg/kg/min in each group. To demonstrate a similar difference as being statistically significant at the 5% level, with 90% power, 33 patients were required per group.

7.3.3.8.2.3. The 6MWT

Cardiac rehabilitation has been demonstrated to improve the 6MWT by 46% in patients following CABG surgery from 281 metres to 411 metres (564). We conservatively expected to demonstrate a difference of a third of this magnitude (i.e. 15%). The mean difference would thus be 42 metres with a pooled SD of 99 metres. To demonstrate such a difference as being statistically significant at the 5% level, with 90% power, 117 subjects were required per group.

7.3.3.8.2.4. Quality of life

The sample size of 770 patients per treatment group was sufficient to detect even small effects of RIPC on quality of life at the 12-month follow-up. With 770 patients per group, a 90% power could detect an effect size of 0.2 in the HRQOL as statistically significant at the 5% level. This effect size is similar to that found for studies of pacemaker implantation and is at the lower limit of a clinically worthwhile difference (567).

7.3.3.8.235. Echo substudy

In a previous study, RiPostC was reported to improve LVEF by 7% from 49% to 56% at one year in STEMI patients (568). In order to detect a smaller mean difference of 5% with a common SD of 10.5%, our substudy required 70 patients in each group using 80% power and a 5% significance level.

7.3.3.9. Study duration and timetable

The anticipated duration of the study was 48 months, distributed as follows:

1) **0-6 months.** Study preparation to obtain:
   - Ethical and Research &Department approval for each recruiting centre (0-2 months).
   - Staff recruitment (advertising, interviews and training) (0-2 months).
   - Research protocol publication. Staff training at the 30 recruiting centres (4-6 months).
2) **6-30 months.** Patient recruitment (1610 patients in 30 centres) over 24 months
3) **7-32 months**. Six week follow-up with:
   - Assessment of clinical outcomes.
   - 6MWT
   - Blood test for creatinine
   - HRQOL questionnaire
   - ECG
4) **9-39 months.** 3, 6, 9 month HRQOL questionnaire
5) **18-42 months**. One year follow-up with:
   - Assessment of clinical outcomes.
   - SMWT
   - Blood test for creatinine
   - HRQOL questionnaire
   - ECG
   - Echocardiography (substudy)
6) **42-48 months**. Data analysis, with:
   - Closing the database, data cleaning and analysis of the data.

- Time for production of the draft report.
- Dissemination of findings and publication in a peer-reviewed journal.

7.3.3.10. Criteria for Discontinuation

This may depend on:

- Individual subject
  - Patients were free to choose to withdraw from the trial at any time.
  - Operative complications that could directly influence graft revascularisation.
- Unexpected safety issues on the advice of the DCM

7.3.3.11. Data collection

Recruited patients had data collected at the time of consent and in the peri-operative period till hospital discharge (see Appendix).

7.3.3.11.1. Six weeks post-surgery

Patients were reviewed in clinical outpatients' clinic as per normal procedure. ECG and 6MWT were performed and blood tests were taken for creatinine. Patients completed HRQOL questionnaire and information on clinical outcomes was recorded.

7.3.3.11.2 Three, Six and Nine months post-CABG with or without valve surgery

Patients were contacted and the HRQOL questionnaire completed over the telephone or sent by post/e-mail to complete and return.

7.3.3.11.3. One-year post-surgery

Patients are reviewed in research outpatient clinic by research nurse, as most surgical centres do not routinely follow-up post-surgery at one year. The general practitioner (GP) and hospital medical notes will be reviewed regarding any major cardiac or cerebral events. If the patient is unable to attend an outpatient clinic appointment the one-year follow up may be conducted over the telephone. In addition, the following information will be taken:

- Weight
- Heart rate
- Blood Pressure
- Recording of primary endpoints (see Appendix)
- ECG
- Blood test taken for creatinine
- TTE to assess LVEF (substudy)
- 6MWT will be performed
- HRQOL questionnaire.

Analysis of data will be checked and information recorded. Patient will therefore be discharged from the clinical study at one year. A summary of the study procedure schedule is given in **Table 7.2.**

The trial shall be considered finished when the last patient recruited reaches the 1-year follow up point. At that point notification of closure of the study will be sent to the Research Ethics Committee.

Table 7.2. Study procedures table

	Pre-operative period		Operation	Post-operative in hospital				Outpatients				
	Screen	PAC Or IP	Day 0	Post-op Day 1	Post-op Day 2	Post-op Day 3	Discharge	6 wks	3 mths	6 mths	9 mths	12 mths
Clinical Assessments												
Informed consent		X										
Review of inclusion/ exclusion criteria		X										
History and examination		X										
Inotrope score			X	X	X	X						
ICU stay							X					
Hospital stay							X					
Six min walk test		X						X				X
Echo (substudy)		X										X
QOL questionnaire		X						X	X	X	X	X
Laboratory Assessments												
Creatinine			X	X	X	X		X				X
hsTnT			X Pre-op, 6, 12 hr	X 24hr	X 48hr	X 72hr						
NGAL			X Pre-op, 6,12 hr	X 24hr								
Urine volumes			X	X	X	X						
Proteomics		X Pre-op	X PostRIC									
Clinical outcomes												
Death							X	X				X
MI							X	X				X
Revascularisation							X	X				X
Stroke							X	X				X

PAC=pre-admission clinic; IP=in-patient; ICU=intensive care unit; QOL=quality of life; hsTnT=high sensitivity troponin-T; NGAL=neutrophil gelatinase lipocalin; MI=myocardial infarction

7.3.3.12. Assessment of Safety

Crucially, our study is not a trial of an investigational medicinal product. Therefore, by definition all untoward occurrences are adverse events rather than adverse reactions. Safety assessments will be from time of randomisation to completion of follow up: an adverse event is any untoward medical occurrence affecting a patient, which does not necessarily have a causal relationship with the RIPC stimulus. The terms *"mild, moderate or severe"* are used to describe the intensity of a specific event or reaction, which is not the same as *"serious"* (see below). An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of RIPC whether or not considered related to the technique.

7.3.3.12.1. Serious adverse event (SAE)

A serious adverse event (SAE) is any untoward medical occurrence/effect that:

1. Results in death.
2. Is life-threatening.
3. Requires hospitalisation or prolongation of existing inpatient's hospitalisation.
4. Results in persistent or significant disability or incapacity.

"Life-threatening" in the definition of a serious adverse event refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event, which hypothetically might have caused death if it was more severe.

7.3.3.12.2. Unexpected adverse event

This is defined as an adverse event, the nature or severity of which is not consistent with an expected consequence of RIPC.

7.3.3.12.3. Expected adverse events (recognised to be caused by the RIPC stimulus)

The benign nature of the RIPC stimulus excluded there would be expected SAEs. Skin petechiae caused by cuff inflation were expected to be the only non-serious events in response to the RIPC stimulus and were be recorded on the Case Report Form (CRF).

7.3.3.12.4. Expected SAEs related to usual clinical care

These events are recognised complications of:

1) CABG with or without valve surgery. They were recorded on the CRF but did not need to be reported separately.
    - Death, PMI or MI.
    - Acute renal failure, requiring haemodialysis, peritoneal dialysis, or haemofiltration.
    - AF.
    - Significant heart block requiring temporary or permanent cardiac pacing.
    - Bleeding requiring re-do surgery.

2) Complications of surgery:
    - Bowel obstruction
    - Sepsis
    - Gastro-intestinal bleed or haematemesis
    - Chest infection
    - Respiratory failure
    - Respiratory tract infection
    - Pleural effusion
    - Urinary tract infection

- Pulmonary embolism
- Atrial flutter
- Infection of donor site

3) Complications due to administration of anaesthetic agents.

4) Known adverse effects of other drugs used in routine clinical care.

7.3.3.12.5. Unexpected adverse events

Investigators will make their reports of all unexpected adverse events, whether serious or not, to the Clinical Trials Unit, LSHTM. SAEs are those not described in the previous section and should be reported with an assessment of causality by the Principal Investigator at each site. The Chief Investigator will be responsible for the prompt notification of findings that could adversely affect the health of subjects or impact on the conduct of the trial. Notification of confirmed unexpected SAEs will be to the Sponsor, the Research Ethics Committee and the DMC. All deaths will be reported to the sponsor irrespective of whether the death is related to cardiac surgery or is an unrelated event.

Unexpected non-serious adverse events should be evaluated by the Principal Investigatorr with an assessment of causality and intensity. The Clinical Trials Unit will keep detailed records of all unexpected adverse events reported. Reports will be reviewed by the Chief Investigator to consider intensity, causality, and expectedness. As appropriate these will be reported to the sponsor, the DMC and the Ethics Committee. The intensity will be classified as:

- *Mild:* the subject is aware of the event or symptom, but this is easily tolerated.
- *Moderate:* the subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity.

- *Severe:* the subject is unable to carry out usual activities and/or his/her life is at risk from the event.

With regards to the relationship of causality, this will be**:**

- Probable: a causal relationship is clinically/biologically highly plausible and there is a plausible time sequence between onset of the adverse event and administration of the intervention.
- Possible: a causal relationship is clinically/biologically plausible and there is a plausible time sequence between onset of the adverse event and administration of the intervention.
- Unlikely: a causal relationship is improbable and another documented cause of the adverse event is most plausible.
- Unrelated: a causal relationship can definitely be excluded and another documented cause of the adverse event is most plausible.

7.3.3.13. Research Governance

The nominated sponsor of our research study is UCL.

Additionally a number of committees are in place in order to ensure the study validity:

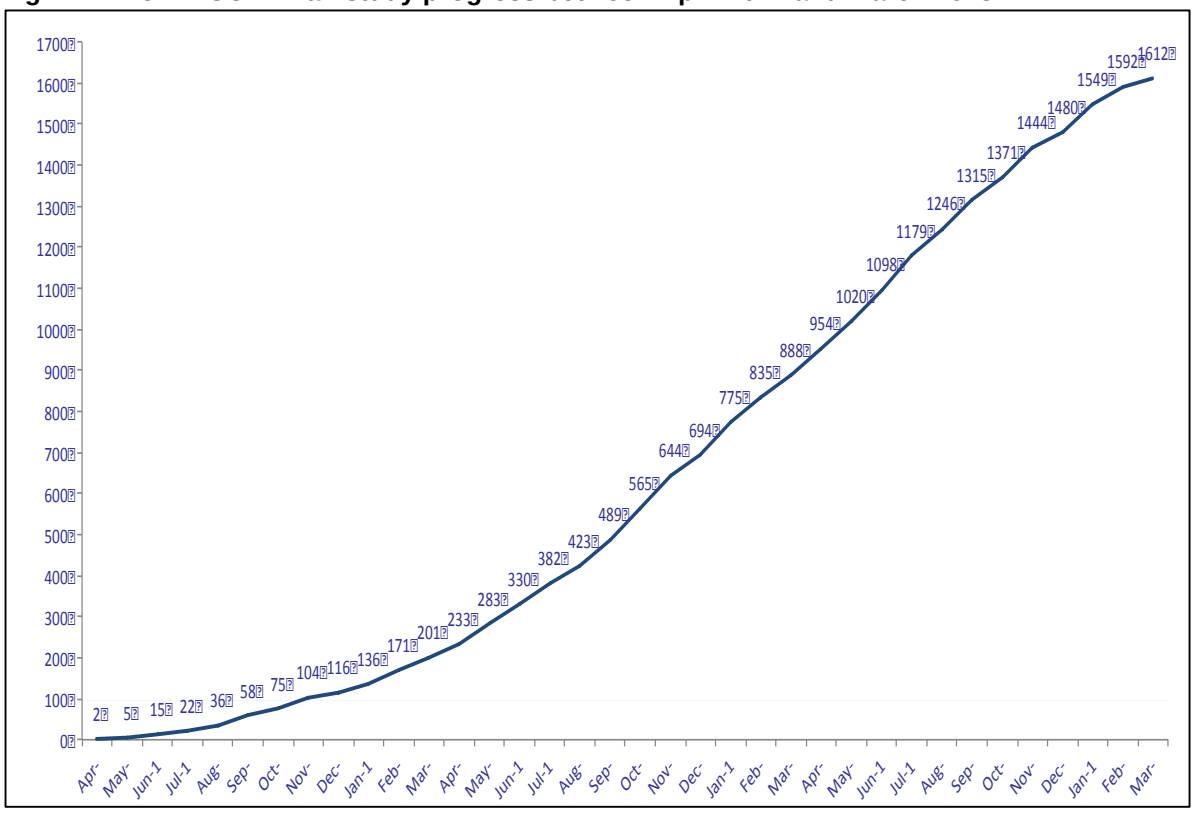
1. Trial Steering Committee (TSC), meeting every 6 months and responsible for drafting the final report and submission for publication.
2. Program Management Group (PMG), meeting weekly during the planning stages of the study and less frequently during recruitment.
3. DMC, meeting at the start of the trial to establish a DMC charter then at 24, 36 and 48 months to determine if there are any unforeseen effects of RIPC.
4. Endpoint validation committee (EVC), meeting quarterly to validate and adjudicate primary endpoints.

7.4. Preliminary results

Patients' recruitment started in April 2011 at the Heart Hospital and then gradually progressed across the 30 participating centres in the UK: it was completed in March 2014 when the last patient was enrolled to the study for a total of 1612 subjects recruited. An extension of the recruitment period was necessary in order to enrol the required number of patients as per sample size calculation. This also implies that the last outpatient follow-up will be due in March 2015 **(Fig. 7.2)**.

During these two years I have been involved in PMG and TSC meetings. I have been the clinical lead for the study at the Heart Hospital, the leading centre for the ERICCA trial. I have contributed to protocol changes and liaised with members from cardiothoracic and anaesthetic teams in order to ensure appropriate running of the study at all the participating centres.

Fig. 7.2. The ERICCA Trial: study progress between April 2011 and March 2013



I have been responsible for site visit initiations across the UK and staff recruitment and coordinated regular meetings with biochemists to guarantee appropriate collection and storage of blood samples. I have been the reference point for research nurses and colleagues when question arose regarding the different aspects of the study. I have adjusted the Case Report Form as appropriate and prepared Standards of Procedures on hsTnT, NGAL, creatinine, biomarkers and 6MWT (see Appendix). Crucially I have been the unblinded member of the team at the Heart hospital and I have written the relevant published peer-reviewed paper (290). I recruited the very first study patients at the Heart Hospital: our clinical trial then progressed to recruit the expected number of patients with variable rates of enrolment across the different centres **(Figures. 7.3-7.4).** The Adjudication committee is now meeting regularly: we intend to disseminate the study results in 2015.

Fig. 7.3. Recruitment by centre

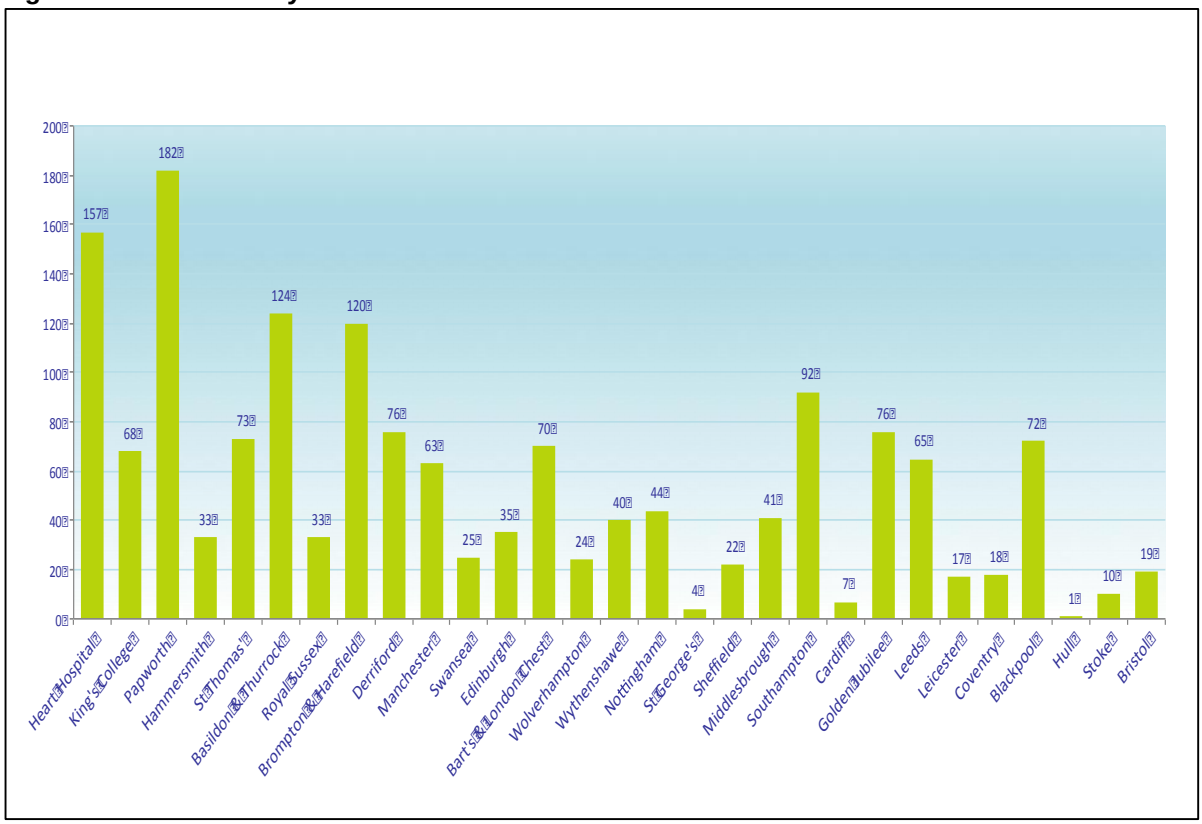
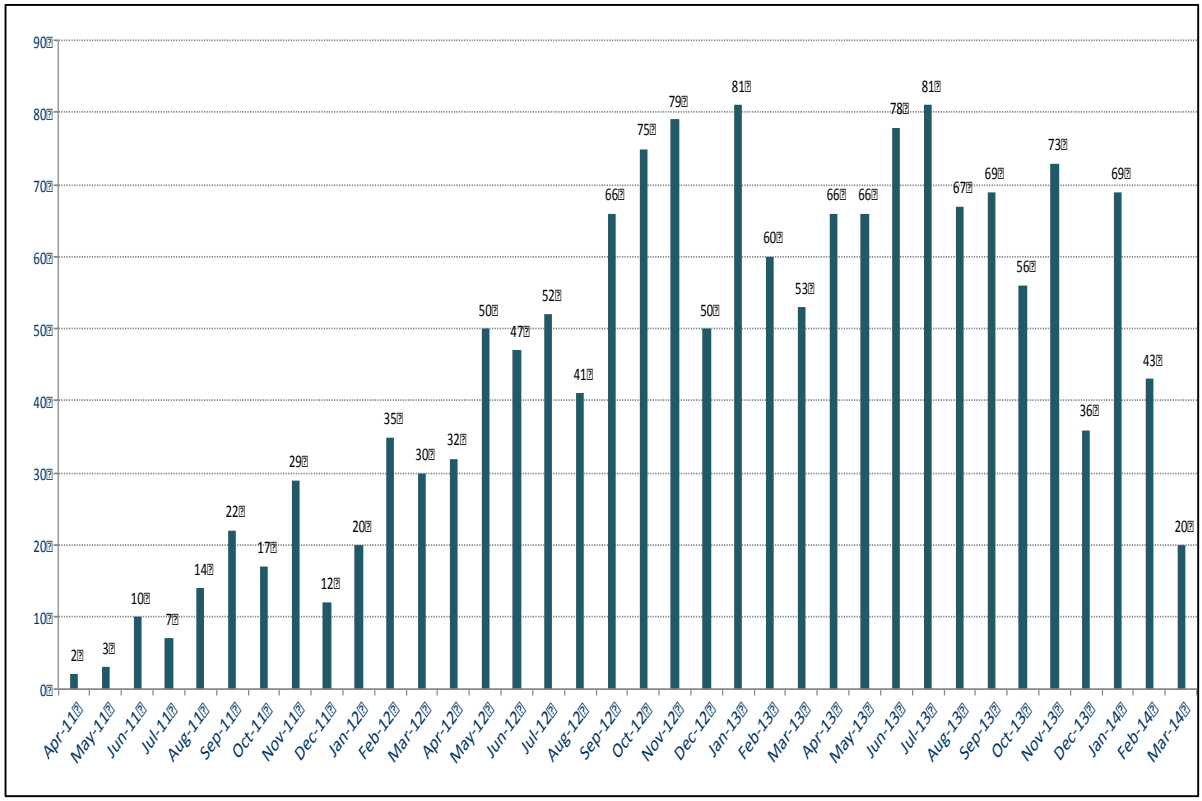


Fig. 7.4. Recruitment by month



7.5. Discussion

The risk profile of patients undergoing CABG surgery is progressively increasing given the aging population, the growing prevalence of diabetes and other significant co-morbidities, and the more advanced complexity of cardiac surgery (34). This has resulted in an increase of operative risk of approximately 5-6% (33, 34) with a subsequent augmented potential to sustain PMI, AKI (557), stroke (558) and to therefore result in worse short and long-term clinical outcomes. RIPC has been demonstrated to be a simple, non-invasive, risk-free intervention potentially able to protect the myocardium and other organs from IRI (282, 284, 285, 291, 293-299, 302-306, 445, 469) (see chapter 1). In our ERICCA trial we have specifically selected

higher-risk patients as these subjects represent the group which more significantly requires optimisation of protective strategies, therefore differently from previously published studies where no EuroSCORE was clearly documented or patients had a lower mean value in the vast majority of cases **(Table 7.1)**. In line with the rationale of my single-centre study, we also decided to increase the intensity of our preconditioning stimulus to four-5 minutes cycles of upper arm IR in order to maximise cardioprotection and to overcome potential resistance of the diabetic myocardium and interference from other factors including volatile anaesthetics. Moreover, the same preconditioning stimulus had successfully been applied to patients presenting with STEMI and undergoing PPCI (356).

Crucially, the vast majority of RCTs investigating the effects of RIPC are relatively small proof-of-concept studies and have often given discordant results for potential reasons, which we have comprehensively elucidated in chapter 3. At the time of the ERICCA initiation, a significant part of these studies endpoints included surrogate endpoints such biochemical assessments of PMI through serial evaluation of serum levels of cardiac enzymes, yet very little data are available in literature with regards to the potential beneficial effects of RIPC on short and long-term clinical outcomes **(Table 7.1).** The very first study to describe the impact of RIPC on patients' morbidity and mortality in the context of cardiac surgery reported no post-operative death in either preconditioned or control patients 30 days after elective AVR, MV surgery or double valve replacement (301). Similarly, no significant difference in major cardiac and cerebro-vascular events was found at 30 days post-operatively in two small studies involving patients undergoing elective CABG surgery with crystalloid cardioplegia (293, 297). Interestingly, in a study including high-risk patients (296) (mean EuroSCORE of 7.1±6.1 in the RIPC group and 6.6±6.1 in the control group,

p>0.05), again no difference in mortality rate was found at 30 days post-surgery. However, an improvement of NYHA functional status and mean LVEF at 3 months post-operatively was found in preconditioned patients undergoing elective valve replacement (304). Additionally, RIPC, given with four-5 minutes cycles of lower limb IR to patients undergoing CABG surgery under strict anaesthetic regime (299), was associated with a higher peri-operative composite end-point of new arrhythmias and new MI, yet no significant difference was found at 6 months follow-up.

Crucially, in the so-far largest proof-of-concept clinical trial investigating the effects of RIPC in the context of elective CABG surgery, unpublished at the time of the initiation of the ERICCA trial, Thielmann et al (469) reported a statistically significant improvement of all-cause mortality and MACCE rate in preconditioned patients at 1 year and at follow-up completion (1.54+/-1.22 years), which was mainly driven by reduced incidence of new MI, whereas no significant difference was found in the occurrence of cardiac death, stroke and repeat revascularisation. Interestingly, of the 329 patients randomised and included in the intention-to-treat analysis, 71 were excluded of which 61 with known DM, and therefore the final in per-protocol analysis comprised a total of 258 subjects. However, the study was a single-centre trial and adequately powered for the primary endpoint of PMI but not for secondary end-points including clinical outcomes and crucially, despite still lower, the rate of all-cause mortality became non-statistically significant when deaths from sepsis were excluded.

In addition, a significant number of systematic reviews and meta-analyses in patients undergoing cardiac or vascular surgery or elective PCI have been conducted (289, 446-456): the overall conclusion confirmed the beneficial effects of RIPC on PMI reduction, however no statistically significant improvement of clinical outcomes was

observed, including the rate of death, peri-operative MI, renal failure, stroke, mesenteric ischaemia, hospital or ICU stay.

In our single centre RCT, we could not demonstrate that simultaneous multi-limb preconditioning reduces the rate of death, MI, revascularisation and stroke at 6 weeks post-cardiac surgery, a finding, which was also confirmed in the subsequent subgroup analyses: however, once again the study was not powered for this type of evaluation.

Our ERICCA study is a multi-centre randomised control double-blinded clinical trial which we have demonstrated to be adequately powered for the primary endpoint as well as for each of the secondary end-points described: results from this study will therefore be able to determine whether RIPC can improve clinical outcomes at 1-year in high-risk patients undergoing CABG with or without valve surgery and have the crucial potential to change clinical practice with the introduction of a non-invasive and risk-free intervention.

Chapter 8

8. Conclusions and future considerations

8.1. Effects of multi-limb RIPC on cardioprotection

With our single-centre single-blinded RCT we have demonstrated that an enhanced simultaneous multi-limb RIPC stimulus reduces total PMI in an unselected cohort of patients undergoing elective cardiac surgery, as demonstrated by a statistically significant reduction of total hsTnT AUC **(Table 8.1)**. This confirms our hypothesis that an enhanced preconditioning stimulus is able to confer cardioprotection in the context of cardiac surgery, where a number of factors, including patients' age, concomitant co-morbidities and pharmacotherapy, clinical setting, type of surgery, technique of myocardial preservation, anaesthetic agents and intraoperative GTN, are able to interfere with the protective mechanisms of RIPC. It also confirms our suggestion that the intensity of the preconditioning stimulus represents one of the key factors of RIPC-induced cardioprotection and therefore a potential reason for the negative outcomes of recently published RCTs. Our trial included a total of 178 patients and to our knowledge it was at the completion of recruitment the largest proof-of-concept study in this field. It was presented at the Conference of the British Cardiovascular Society (BCS) and at the Annual Congress of the Society of Cardiothoracic Surgery in 2013. In addition, my abstract with the relevant outcomes of the study obtained the BCS first prize in the "Stable IHD/Prevention/Hypertension category". Results from our RCT were published in the Heart Journal (569).

Table 8.1. Effects of RIPC on PMI: study outcomes

Type of surgery	Group	72-hours AUC (µg/L) (SD)	Difference (95% CI)	P value
Unselected cohort	Control: n=89 RIPC: n=89	36.307(24.542) 27.004 (16.523)	-9.303 (-15.626, -2.979)	0.004
All surgery with Cardioplegia	Control n=73 RIPC: n=75	37.089 (25.730) 27.942 (17.386)	9.146 (1.861, 16.433)	0.014
All surgery with ICCF	Control n=16 RIPC: n=14	32.885 (18.771) 20.692 (6.039)	12.192 (0.066, 24.319)	0.049
CABG±valve surgery	Control: n=64 RIPC: n=66	33.526 (20.164) 24.772 (12.640)	8.753 (2.808, 14.688)	0.004
CABG±valve surgery with cardioplegia	Control: n=48 RIPC: n=52	33.74 (20.81) 25.64 (13.52)	8.11 (1.01, 15.21)	0.026
CABG surgery alone	Control: n=54 RIPC: n=57	30.753 (18.948) 23.609 (12.004)	7.14 (1.076, 13.21)	0.022
CABG surgery with cardioplegia	Control: n=38 RIPC: n=43	29.832 (19.206) 24.355 (13.052)	5.477 (-1.985, 12.938)	0.147
Valve surgery alone	Control: n=25 RIPC: n=23	43.925 (33.144) 33.395 (23.719)	10.529 (-6.868, 27.927)	0.229
AVR alone	Control: n=15 RIPC n=14)	38.499 (37.661) 27.947 (24.678)	10.55 (-14.96, 36.06)	0.402
All surgery with GTN	Control: n=65 RIPC: n=21	30.81 (17.56) 26.69 (13.93)	4.12 (-1.92, 10.17)	0.179
All surgery without No-GTN	Control: n=53 RIPC: n=35	50.52 (34.20) 27.86 (20.01)	22.66 (8.03, 37.29)	0.003
All surgery with DM	Control: n=24 RIPC: n=28	35.993 (21.859) 25.927 (20.031)	10.07 (-1.844, 21.00)	0.096
All surgery with Non-DM	Control: n=65 RIPC: n=61	36.428 (25.673) 27.479 (14.899)	8.949 (1.423, 16.477)	0.020
All surgery + cardioplegia with DM	Control: n=18 RIPC: n=23	34.88 (20.576) 26.59 (21.23)	7.51 (0.066, 21.029)	0.216
All surgery + cardioplegia with no DM	Control: n=55 RIPC: n=52	37.85 (27.43) 28.55 (15.55)	9.30 (0.58, 18.02)	0.037
CABG surgery +DM	Control: n=17 RIPC: n=19	31.73 (18.63) 19.63 (9.19)	12.09 (1.83, 22.35)	0.022
CABG surgery + NON-DM	Control: n=37 RIPC: n=38	30.29 (19.34) 25.43 (12.79)	4.86 (-2.84, 12.56)	0.212
CABG surgery with cardioplegia + DM	Control: n=11 RIPC: n=27	27.59 (11.46) 19.37 (10.03)	8.22 (-0.68, 17.11)	0.069
CABG surgery with cardioplegia + NON-DM	Control: n=14 RIPC: n=29	30.78 (21.81) 26.76 (13.79)	4.02 (-5.74, 13.78)	0.413

AUC=area-under-the curve; SD=standard deviation; CI=confidence interval; RIPC=remote ischaemic preconditioning; ICCF=intermittent cross-clamp fibrillation; CABG=coronary artery bypass graft; AVR=aortic valve replacement; GTN=glyceryl trinitrate; DM=diabetes mellitus

Furthermore, we conducted a series of retrospective sub-group analyses, which are highly suggestive of further cardioprotective effects of RIPC in specific cohorts of patients: in particularly, RIPC was associated with a statistically significant PMI reduction in patients undergoing:

- cardiac surgery irrespective of the technique of myocardial preservation used;
- CABG with or without valve surgery;
- CABG with or without valve surgery using cardioplegia;
- CABG alone;
- cardiac surgery when no GTN was used intra-operatively.

This is the first study to demonstrate the cardioprotective effects of RIPC in the setting of ICCF, as only one study previously showed PMI reduction with IPC in patients undergoing CABG surgery (468). Furthermore, we found no significant difference between AUC of control patients receiving cardioplegia or ICCF, confirming previous studies showing that the two techniques are associated with comparable PMI magnitude. It is also the first clinical trial to show a significant impact of intravenous administration of nitrates in patients undergoing elective cardiac surgery.

With respect to DM, we also found that RIPC was cardioprotective in diabetic subjects undergoing CABG and in non-diabetic patients undergoing any cardiac surgery. In all other subgroups RIPC led to a total hsTnT AUC reduction, which however did not reach statistical significance. This confirms previous findings (440) that cardioprotection in the diabetic myocardium can be achieved with a more potent preconditioning stimulus able to overcome the higher threshold required in this

condition. In contrast with previous literature, we found that the level of PMI sustained by our control diabetic patients was not different from that of non-diabetic controls.

In a further subgroup analysis of control CABG patients, we showed that combined antegrade and retrograde cardioplegia conferred more significant cardioprotection compared to antegrade cardioplegia alone and ICCF alone: this is to our knowledge the first analysis combining four factors such as the aortic cross-clamping times, combined antegrade/retrograde versus antegrade cardioplegia alone versus ICCF, hsTnT levels at 6 different time-points with total hsTnT-AUC, and exclusively CABG patients. The results of this study have been accepted for publication on the Journal of Cardiothoracic Society (see Appendix).

8.2. Effects of multi-limb RIPC on secondary outcomes

Our study comprised a number of secondary endpoints such incidence of post-operative AKI and AF, inotrope requirement and length of ICU and hospital stay. RIPC significantly reduced the incidence of AKI by 71% in patients undergoing cardiac surgery and not administered GTN intra-operatively. Furthermore, it was also associated with a significant decrease of the rate of new post-operative AF by 55% in unselected cohort of patients, 37% in patients undergoing CABG with or without valve surgery, 60% in those, within this group, receiving cardioplegia only, and in subjects having CABG alone, 83% in diabetics undergoing CABG only.

Importantly, RIPC reduced the duration of ICU stay by 1 day in the unselected cohort of patients, in subjects undergoing CABG with or without valve surgery and in those within this group receiving cardioplegia only. The duration of hospital stay was

also reduced by 1.5 days in preconditioned patients undergoing CABG with or without valve surgery with cardioplegia.

In addition, we also intended to evaluate whether the simultaneous inflation of two blood pressure cuffs, one around the upper arm, one around the thigh, in order to induce our enhanced preconditioning stimulus was associated with a more significant level of skeletal muscle injury in preconditioned patients compared to control. We found no significantly difference in total AUC between the two intervention groups in either the unselected cohort of any of the subgroups we then went on to analysis retrospectively. We therefore demonstrated that the enhanced preconditioning stimulus proposed in our study can be safely applied without any significant increase of muscle injury.

8.3. Limitations

Our study presented a series of limitations, which are partly in common with the vast majority of clinical trials investigating the effects of RIPC in the context of cardiac surgery. Firstly, our patient recruitment took place at a single centre over almost a two year-period: therefore, despite the significant number of patients enrolled (n=178), the cohort size was still relatively small and consequently study outcomes will need to be taken with careful consideration. In particular, our study was not designed - and thereby not adequately powered - for any of the secondary endpoints: this implies that the positive outcomes in AF rate and ICU stay could have been due to chance and only a larger study will be able to confirm these findings. Conversely, AKI incidence remarkably leaned towards statistical significance (p=0.063) and again it is a possible that instead a larger study would also statistically prove the beneficial reno-protective

effects of RIPC. In addition, we obtained extremely intriguing results from the series of retrospective analyses we subsequently conducted, with particular regards to outcomes in diabetic patients, in those who were not administered GTN or in control subjects receiving combined antegrade/retrograde cardioplegia: however, again the study was not sufficiently powered for this.

Secondly, our trial was single blinded and therefore only the member delivering the intervention was aware of patient baseline characteristics and subsequently of surgery details and results available: randomisation was operated via SNOSE system, which contributed to limiting any element of bias. Despite this, whilst the sham protocol involved leaving uninflated blood pressure cuffs on patient's upper arm and thigh for 15 minutes, it is possible that the anaesthetic and theatre team but not the operating surgeon, could have become aware of the nature of the intervention: however, we strongly doubt that this could have changed patient management at any stage.

Thirdly, our primary end-point was represented by total hsTnT-AUC over the 72 post-operative hours as an indicator of PMI sustained by these subjects: crucially, although troponin and CK-MB post-operative concentrations have been associated with short and long-term patient morbidity and mortality (see chapter 1), hsTnT-AUC remains a surrogate end-point. In our study, we could only establish MACCE rate at the six-week follow-up as this type of trial limited any further follow-up.

A final drawback of our study is given by our selection criteria, which led to the exclusion of patients with severe renal, pulmonary or hepatic disease and of those with recent ACS proved by positive baseline cardiac biomarkers: this is however not a true representation of what happens in the "real world" where instead increasingly complex patients are being operated on.

8.4. On-going clinical trials and future considerations

Our “Effects of Remote Ischaemic Preconditioning on Clinical outcomes in high-risk patients undergoing CABG+/-valve surgery” (ERICCA trial) (290) addressed most of the above-mentioned limitations: in this large multi-centre double-blinded randomised control clinical trial, we recruited a total of 1612 patients across 30 tertiary centre in the UK: these were high risk subjects with an additive EuroSCORE of at least 5 and underwent CABG with or without valve surgery with cardioplegia. In the first instance, the study is adequately powered for the specified primary and secondary end-points, which will make the outcomes robust and objective and with clear statistical evidence. In particular, our primary outcome is a composite of cardiovascular death, non-fatal MI, coronary revascularisation and stroke at one year, which will give a consistent evaluation of the effects of RIPC on patient morbidity and mortality. Also, the blinding strategy in ERICCA was robust and used an adjustable valve on the cuff rather than a sham arm, in order to give the impression that the cuff was being inflated even in control patients. Crucially we anticipated that the RIPC, given with four-5 minute cycles of upper arm IR, could reduce the event rate from 20% to 12%. I prepared the article on the study design, which was published on the peer-reviewed journal “Clinical research in cardiology” (290)(see Appendix).

Another large multi-centre trial investigating the effects of RIPC on clinical outcomes in cardiac surgery is the “Remote Ischaemic Preconditioning for Heart Surgery study” (RIPHeart-Study)(570), an on-going multi-centre clinical trial in Germany. It aims to recruit 2070 high or low risk patients undergoing cardiac surgery with a standardised cardiopulmonary bypass protocol and with only an intravenous anaesthetic regime in order to eliminate the potential preconditioning effect of volatile anaesthetics. The primary outcome is again a composite of all-cause mortality, non-

fatal MI, new stroke, and/or acute renal failure, although only until hospital discharge (up to a maximum of 14 days after surgery).

Moreover, the Renal Protection Against Ischaemia Reperfusion in Transplantation (REPAIR) trial is another multi-centre trial, aimed to determine the effect of RIPC on renal function after renal transplantation using eGFR at one year as the primary outcome: the study recruited a total of 406 patients and showed that limb RIPC of both donors and recipients was associated with a preserved transplanted kidney function at 6 months in recipients of live-donor related renal transplantation, therefore showing that RIPC is protective on transplanted renal grafts. It also will report on clinical outcomes at 2–5 years using registry follow up.

Other on-going large RCTs investigating the effects of RIPC in setting different from cardio or renal protection have been already discussed in chapter 1.

In conclusion, we have conducted an extremely fascinating and intriguing journey though the different aspects of cardioprotection and RIPC in the context of experimental and clinical studies. These have demonstrated that, despite many barriers encountered by the researchers, with particular regard to the mechanistic pathway and practical application of RIPC in the clinical context, RIPC is a novel, cost effective and widely available protective phenomenon. It has the potential to reduce IRI in major cardiovascular interventions and in many other procedures: biological mechanisms are still partly unknown and methodological issues exist, however RIPC research has advanced considerably over the recent decades and the future challenge will be to clarify the mechanistic pathways and demonstrate substantial benefits in patient outcomes.

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APPENDIX

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ORIGINAL ARTICLE

Effect of remote ischaemic preconditioning on clinical outcomes in patients undergoing cardiac bypass surgery: a randomised controlled clinical trial

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ABSTRACT

Objectives Remote ischaemic preconditioning (RIPC), using brief cycles of limb ischaemia/reperfusion, is a non-invasive, low-cost intervention that may reduce perioperative myocardial injury (PMI) in patients undergoing cardiac surgery. We investigated whether RIPC can also improve short-term clinical outcomes.

Methods One hundred and eighty patients undergoing elective coronary artery bypass graft (CABG) surgery and/or valve surgery were randomised to receive either RIPC (2–5 min cycles of simultaneous upper arm and thigh cuff inflation/deflation; N=90) or control (uninflated cuffs placed on the upper arm and thigh; N=90). The study primary end point was PMI, measured by 72 h area under the curve (AUC) serum high-sensitive troponin-T (hsTnT); secondary end point included short-term clinical outcomes.

Results RIPC reduced PMI magnitude by 26% (−9.303 difference (CI −15.618 to −2.987) 72 h hsTnT-AUC; p=0.003) compared with control. There was also evidence that RIPC reduced the incidence of postoperative atrial fibrillation by 54% (11% RIPC vs 24% control; p=0.031) and decreased the incidence of acute kidney injury by 48% (10.0% RIPC vs 21.0% control; p=0.063), and intensive care unit stay by 1 day (2.0 days RIPC (CI 1.0 to 4.0) vs 3.0 days control (CI 2.0 to 4.5); p=0.043). In a post hoc analysis, we found that control patients administered intravenous glyceryl trinitrate (GTN) intraoperatively sustained 39% less PMI compared with those not receiving GTN, and RIPC did not appear to reduce PMI in patients given GTN.

Conclusions RIPC reduced the extent of PMI in patients undergoing CABG and/or valve surgery. RIPC may also have beneficial effects on short-term clinical outcomes, although this will need to be confirmed in future studies.

Trial registration number ClinicalTrials.gov ID: NCT00397163.

INTRODUCTION

Coronary artery bypass graft (CABG) surgery is the revascularisation strategy of choice for patients with multivessel coronary artery disease. Higher-risk patients are being operated on for a number of different reasons, including the aging population, the presence of comorbidities such as diabetes, obesity and hypertension, and the increasing incidence of concomitant valve surgery. These higher-risk patients are more susceptible to perioperative myocardial injury (PMI) and experience worse short-term and long-term clinical outcomes.[1] Therefore, novel therapeutic interventions are required to protect the heart during CABG surgery in these higher-risk patients in order to improve patient morbidity and mortality.

In this regard, remote ischaemic preconditioning (RIPC), in which the application of one or more brief cycles of non-lethal ischaemia and reperfusion to an organ or tissue protects the heart against a lethal episode of acute ischaemia-reperfusion injury (IRI),[2 3] has emerged as a non-invasive, low-cost therapeutic intervention for potentially reducing the extent of PMI (as measured by serum cardiac enzymes) in patients undergoing CABG and/or valve surgery.[4–16] The majority of these clinical studies have reported beneficial effects using a standard single-limb RIPC protocol comprising three or four 5 min cycles of inflation and deflation of a cuff placed on either the upper arm or thigh to induce transient ischaemia. However, several recent studies have failed to demonstrate any reduction in PMI using this standard single-limb RIPC stimulus, suggesting that under certain conditions this RIPC stimulus may be ineffective.[6 10 17]

Whether increasing the intensity of the RIPC stimulus by simultaneously applying the RIPC protocol to the upper arm and thigh is more effective in patients undergoing CABG and/or valve surgery is unknown and is investigated in this study. Furthermore, whether RIPC can improve short-term clinical outcomes in this patient group is unknown and is explored here.

METHODS

Study design

This double-blinded randomised controlled clinical trial received local University College London Hospitals (UCLH) Ethics Committee approval and was conducted at the UCLH Heart Hospital (London, UK), in accordance with UCLH guidelines. Written informed consent was obtained from all patients recruited into the study. Randomisation was carried out using a computer-generated list of randomised numbers, and allocation concealment obtained using Sequentially Numbered Opaque Sealed Envelopes.

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Randomisation, treatment allocation and delivery of RIPC or control protocols were performed by an unblinded investigator not involved in data collection or analysis. The investigator collecting and analysing the data, patients, cardiac surgeons and anaesthetists, operating theatre staff and staff on intensive care unit (ICU) and cardiac wards were all blinded to treatment allocation.

Inclusion and exclusion criteria

We recruited adult patients (>18 years of age) undergoing on-pump CABG and/or valve surgery at the UCLH Heart Hospital between December 2010 and July 2012. Patient exclusion criteria were cardiogenic shock or cardiac arrest in the current hospital admission; positive baseline serum hsTnT; pregnancy; significant peripheral arterial disease affecting upper and/or lower limbs; significant hepatic (INR>2.0), pulmonary (forced expiratory volume-1<40% predicted) or renal disease (estimated glomerular filtration rate <30 mL/min/1.73 m^2); and concomitant therapy with glibenclamide or nicorandil, as these medications may interfere with RIPC.

Intervention

RIPC and control protocols were initiated after anaesthesia induction and completed prior to sternotomy. RIPC was delivered with one standard blood pressure cuff placed on the upper arm and another standard blood pressure cuff placed on the upper thigh. The cuffs were then simultaneously inflated to 200 mm Hg and left inflated for 5 min, then deflated to 0 mm Hg and left uninflated for 5 min. This cycle was repeated twice so that the total duration of the RIPC protocol was 20 min. If the systolic blood pressure was >185 mm Hg, the cuffs were inflated to 15 mm Hg above that level. For the control protocol, the two cuffs were placed on the upper arm and the upper thigh and left uninflated for 20 min.

Surgical procedure

Patients received premedication with oral temazepam 10–20 mg 1 h prior to surgery. Anaesthesia induction was achieved with different combinations of midazolam, etomidate, propofol, fentanyl and antinicotinic agents (rocuronium, vecuronium or pancuronium). The trachea was intubated and mechanical ventilation commenced with oxygen with or without air. Anaesthesia maintenance was achieved with volatile agents (isoflurane or sevoflurane) and propofol infusion with or without fentanyl. Arterial blood pressure, central venous pressure, leads I and III of the ECG and nasopharyngeal temperature were recorded continuously. An intravenous glyceryl trinitrate (GTN) infusion, initiated prior to sternotomy and continued until patient transfer to ICU, was administered at the discretion of the anaesthetist at a dose of 25–85 µg/kg/min (titrated to blood pressure). Standard non-pulsatile cardiopulmonary bypass (CPB) was employed using a membrane oxygenator and cardiotomy suction: following this, all coronary grafts were constructed during CPB using either intermittent cross-clamp fibrillation or

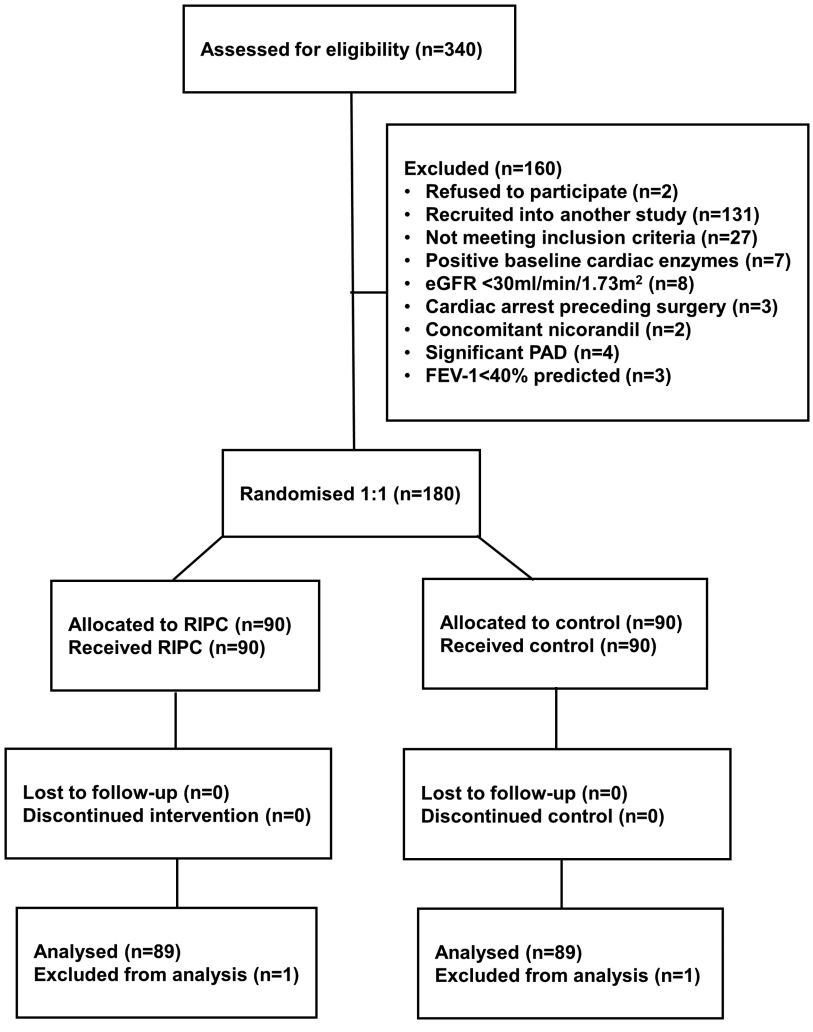

Figure 1 Study profile. RIPC, remote ischaemic preconditioning; eGFR, estimated glomerular filtration rate; PAD, peripheral arterial disease; FEV, forced expiratory volume.

blood cardioplegia. Following anastomosis of the grafts and/or valve replacement/repair, CPB was discontinued and protamine was used to achieve heparin reversal.

Table 1 Patient baseline characteristics

Patients	Control (n=89)	RIPC (n=89)
Age (years)	66±10	65±10
Gender		
Male	67 (75%)	72 (81%)
Female	22 (25%)	17 (19%)
Ethnicity		
Caucasian	74 (83%)	71 (80%)
Asian	10 (11%)	12 (13%)
Afro-Caribbean	4 (5%)	6 (7%)
Chinese	1 (1%)	0 (0%)
BMI	28.4±5.5	28.8±7.1
SBP (mm Hg)	130.0±18.0	129.0±15.7
DBP (mm Hg)	70.7±9.0	70.8±9.4
HR (bpm)	69.2±11.7	66.3±9.8
Smoking history		
Smoker	12 (14%)	11 (12%)
Ex-smoker	52 (58%)	48 (54%)
Non-smoker	25 (28%)	30 (34%)
Family history of IHD	57 (64%)	64 (72%)
NYHA class		
0	8 (9%)	8 (9%)
I	22 (26%)	31 (36%)
II	38 (44%)	39 (46%)
III	17 (20%)	7 (8%)
IV	1 (1%)	0 (0%)
CCS class		
0	30 (35%)	25 (29%)
I	17 (20%)	19 (22%)
II	30 (35%)	30 (35%)
III	7 (8%)	9 (11%)
IV	2 (2%)	2 (2%)
LVEF		
>50%	70 (79%)	67 (75%)
30%–50%	17 (19%)	16 (18%)
<30%	2 (2%)	6 (7%)
Comorbidities		
Diabetes mellitus	24 (27%)	28 (32%)
Hypertension	70 (79%)	65 (73%)
Hypercholesterolaemia	64 (72%)	68 (76%)
Atrial fibrillation	16 (18%)	10 (11%)
Previous MI	23 (26%)	28 (32%)
Previous PCI	11 (12%)	11 (12%)
Previous CVA/TIA	9 (10%)	5 (6%)
Previous cardiac surgery	2 (2%)	4 (5%)
Other comorbidities	35 (40%)	32 (36%)
Peripheral arterial disease	6 (7%)	1 (1%)
Drug history		
Aspirin	66 (76%)	72 (84%)
Clopidogrel/prasugrel	27 (31%)	24 (28%)
Warfarin	9 (10%)	6 (7%)
Beta-blocker	55 (63%)	57 (66%)
Calcium channel blocker	32 (37%)	22 (26%)
Statin	72 (83%)	72 (84%)
ACE-I/ARB	61 (70%)	57 (66%)
Long-acting nitrates	14 (16%)	12 (14%)
Antidiabetics		
Insulin	7 (8%)	8 (9%)
Biguanide	16 (18%)	19 (22%)
Sulfonylurea	11 (13%)	7 (8%)
Diuretics	27 (31%)	31 (36%)

Data are mean±SD.
The following characteristics have missing values: BMI (control 2, RIPC 2), SBP (control 2, RIPC 2), DBP (control 2, RIPC 2), HR (control 2, RIPC 2), NYHA (control 3, RIPC 4), CSS (control 3, RIPC 4), other comorbidities (control 1, RIPC 1), aspirin (control 2, RIPC 3), clopidogrel/prasugrel (control 2, RIPC 3), warfarin (control 2, RIPC 3), beta-blocker (control 2, RIPC 3), calcium channel blocker (control 2, RIPC 3), statin (control 2, RIPC 3), ACE-I/ARB (control 2, RIPC 3), long-acting nitrates (control 2, RIPC 3), insulin (control 2, RIPC 3), biguanide (control 2, RIPC 3), sulfonylurea (control 2, RIPC 3), diuretics (control 2, RIPC 3).
ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCS, Canadian Cardiovascular Society; CVA, cerebrovascular accident; DBP, diastolic blood pressure; HR, heart rate; IHD, ischaemic heart disease; INR, international normalised ratio; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NYHA, New York Health Association; PCI, percutaneous coronary intervention; RIPC, remote ischaemic preconditioning; SBP, systolic blood pressure; TIA, transient ischaemic attack.

Study primary end point

The study primary end point was PMI, assessed by measuring the total 72-hour area under the curve (AUC) hsTnT. Blood samples for hsTnT were taken preoperatively and at 6, 12, 24, 48 and 72 h postsurgery: hsTnT was measured quantitatively by a one-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche, Switzerland). This assay can allow detection of concentrations <1.0 ng/L. These assays measure the upper range limit with a coefficient of variation <10%. The threshold level of ≥14 ng/L indicates significant myocardial necrosis.

Study secondary end points

These included the following:

1. *Acute kidney injury (AKI) score*:[18] Serum creatinine and urine output were measured preoperatively and 24, 48 and 72 h postsurgery. AKI was classified with the following grades:
   - Grade 1: serum creatinine rise of >26.4 μmol/L or 150%–200% of baseline and/or urine output <0.5 mL/kg/h for >6 contiguous hours.
   - Grade 2: serum creatinine rise of 200%–300% of baseline and/or urine output <0.5 mL/kg/h for >12 contiguous hours.
   - Grade 3: serum creatinine rise of >300% of baseline or serum creatinine >354 μmol/L with an acute rise of at least 44 μmol/L and/or urine output <0.3 mL/kg/h for >24 h or anuria for 12 h.
2. *Inotrope requirement*,[19] measured every 24 h over the 72 h postoperative period as dosages (μg/kg/min) of

$$(\text{Dopamine} + \text{Dobutamine} + \text{Dopeximine}) + ((\text{Adrenaline} + \text{Noradrenaline} + \text{Isoprotenerol}) \times 100) + [(\text{Enoximone} + \text{Milrinone}) \times 15].$$

3. *Length of ICU and hospital stay*, calculated as the total duration in days of length of stay on ICU and in hospital.
4. *Incidence of postoperative atrial fibrillation (AF)*: This was the incidence of new-onset AF in the first 72 h after surgery

detected by continuous telemetry and ECG (performed by a blinded staff nurse on a daily basis and immediately after the detection of AF on the telemetry, and then analysed by a blinded investigator) and requiring intervention with pharmacological treatment and/or direct current cardioversion.

5. *Major adverse cardiovascular events at 6 weeks*: This was the rate of death, non-fatal myocardial infarction, coronary artery revascularisation and stroke at 6 weeks postoperatively.

Study safety end points

The main study safety end point was skeletal muscle injury from the RIPC protocol (measured by total creatine kinase (CK)-AUC over the first 72 postoperative hours) and any adverse events relating to the RIPC protocol.

Statistical analysis and sample size estimation

Data are presented as mean (SD) or median (IQR). Comparison between treatment groups was made using unpaired Student t test for approximately normally distributed variables or Wilcoxon–Mann–Whitney test for non-normal data. For outcomes collected at different time points, a repeated measures linear regression model was used to estimate the difference at each time point and 95% CIs. Categorical data were analysed using Fisher's exact test. The post hoc analysis of associations between RIPC and GTN was performed using an interaction test in a linear regression model. We hypothesised that RIPC would reduce hsTnT-AUC by a standardised difference of 0.6. At 90% power and significance at the two-sided 5% level, this required a sample size of 60 subjects, which we increased by 33% to accommodate withdrawal or missing data points. A sample size of at least 80 patients per intervention group was determined based on the following assumptions: (a) the largest published study to date on RIPC in PMI,[6] (b) a power of at least 90%, (c) an SD of 0.2 μg/L and (d) type I error rate of 5%.

Analysis was by intention to treat. No adjustment for multiplicity has been applied for secondary outcomes or post hoc analyses. Data were analysed using Stata V.12.1.

RESULTS

We assessed 340 patients for eligibility (see figure 1), of whom 180 patients were enrolled into the study and randomised to receive either RIPC (N=90) or control (N=90): a total of 178 patients were included for final analysis. No significant difference was found between the two treatment groups with respect to baseline patient characteristics (table 1). With regards to the details of surgery, the only evidence of a difference between the two groups was the percentage of patients receiving intravenous GTN, which was higher in the control group (65 vs 53 patients; table 2).

In all patients, the RIPC protocol was completed within an interval period not longer than 45 min prior to sternotomy. There were no untoward consequences or side effects with the RIPC protocol.

RIPC reduced the extent of PMI

The primary end point of total 72 h AUC hsTnT was reduced by 25.6% in patients randomised to receive RIPC compared with control (−9.30 μg/L, 95% CI −15.618 to −2.987, p=0.004; figure 2, table 3). Moreover, baseline preoperative hsTnT levels were <0.02 μg/L and were not significantly different between RIPC and control groups (−0.003 μg/L, 95% CI −0.009 to 0.003), p=0.308; figure 2, table 3). In patients randomised to RIPC, the mean hsTnT was significantly reduced at 6, 12, 24, 48 and 72 h postsurgery compared with control (figure 2, table 3).

Table 2 Details of surgical procedure

Patients	Control (n=89)	RIPC (n=89)
Indication for surgery		
Angina	44 (49%)	40 (45%)
Myocardial infarction	12 (14%)	19 (21%)
Valve disease	23 (26%)	23 (26%)
Angina and valve disease	7 (8%)	4 (5%)
Myocardial infarction and valve disease	1 (1%)	2 (2%)
Infective endocarditis	2 (2%)	1 (1%)
EuroSCORE	3.72±2.03	3.70±2.59
Additive perioperative risk		
Low (EuroSCORE 0–2)	26 (29%)	29 (33%)
Medium (EuroSCORE 3–5)	47 (53%)	38 (43%)
High (EuroSCORE >5)	16 (18%)	22 (25%)
Bypass time (min)	96.7±32.6	89.6±31.0
Cross-clamp time (min)	64.8±26.4	61.5±26.9
Cardioprotection		
Blood cardioplegia	73 (82%)	75 (84%)
Cross-clamp fibrillation	16 (18%)	14 (16%)
Operation		
CABG alone	54 (61%)	57 (64%)
AVR alone	15 (17%)	14 (16%)
CABG+AVR	10 (11%)	9 (10%)
MVR or MV repair	9 (10%)	8 (9%)
AVR+MVR	1 (1%)	1 (1%)
Number of grafts		
One	4 (6%)	5 (8%)
Two	19 (30%)	15 (23%)
Three	29 (45%)	35 (53%)
Four	12 (19%)	11 (17%)
Anaesthetic agents		
Induction		
Antinicotinic agents		
Rocuronium	68 (81.0%)	76 (89%)
Pancuronium	14 (17%)	6 (7%)
Vecuronium	2 (2%)	3 (4%)
Midazolam	45 (54%)	33 (39%)
Etomidate	8 (9%)	7 (8%)
Fentanyl	86 (100%)	85 (100%)
Propofol	76 (88%)	77 (91%)
Maintenance		
Propofol	86 (100%)	85 (100%)
Volatile anaesthetics		
Isoflurane	80 (93%)	81 (95%)
Sevoflurane	6 (7%)	4 (5%)
Intraoperative GTN	65 (76%)	53 (60%)

Values are mean±SEM.
The following procedure variables had missing values: bypass time (control 2, RIPC 1), cross-clamp time (control 4, RIPC 1), antinicotinic agents (control 5, RIPC 4), midazolam (control 5, RIPC 4), etomidate (control 3, RIPC 4), fentanyl (control 3, RIPC 4), propofol on induction (control 3, RIPC 4), propofol during maintenance (control 3, RIPC 4), volatile anaesthetics (control 3, RIPC 4), intraoperative GTN (control 3, RIPC 1).
AVR, aortic valve replacement; CABG, coronary artery bypass graft; EuroSCORE, European System for Cardiac Operative Risk Evaluation; GTN, glyceryl trinitrate; MV, mitral valve; MVR, mitral valve replacement; RIPC, remote ischaemic preconditioning.

RIPC protected kidney function during surgery

The incidence of AKI was decreased in RIPC-treated patients, with 10 new cases of postoperative AKI in the preconditioned

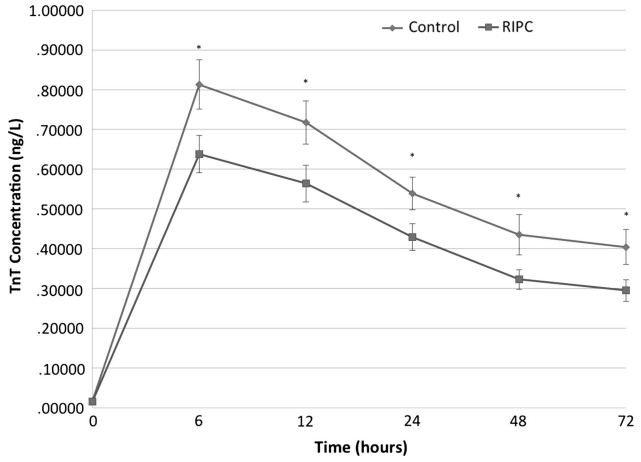

Figure 2 Serum hsTnT levels. Values are mean±SEM. hsTnT, high-sensitivity troponin-T; RIPC, remote ischaemic preconditioning.

group, compared with 19 new cases in the control group, that is, 10.0% versus 21.0% of new cases, which corresponded to a relative reduction of AKI 48% (p=0.063: table 3).

RIPC reduced the incidence of AF and shortened ICU stay

RIPC reduced the incidence of new onset of postoperative AF in the first 72 h postsurgery by 54% (10 RIPC vs 22 control; p=0.03) and decreased the length of ICU stay (RIPC 2.0 days (IQR 1.0 to 4.0) vs control 3.0 days (IQR 2.0 to 4.5); p=0.04) (table 3).

Other end points

Total CK release was not statistically different between control and RIPC patients (32 543±27 087 µg/L control vs 36 312 ±19 496 µg/L RIPC; 3769.6 difference (CI −4647.0 to 12186.2); p=0.38), demonstrating that the multilimb RIPC stimulus was not associated with a significant skeletal muscle injury (table 3). There was no difference in total inotrope

Table 3 Summary of study end points

Endpoint	Control (n=89) (mean (SD))	RIPC (n=89) (mean (SD))	Difference* (95% CI)	p Value*
hsTnT (µg/L)				
Preoperatively	0.018 (0.019)	0.015 (0.020)	−0.003 (−0.099 to 0.093)	
6 h postoperatively	0.802 (0.498)	0.614 (0.381)	−0.188 (−0.285 to −0.092)	
12 h postoperatively	0.709 (0.438)	0.556 (0.376)	−0.153 (−0.250 to −0.057)	
24 h postoperatively	0.529 (0.341)	0.408 (0.268)	−0.124 (−0.221–0.027)	
48 h postoperatively	0.440 (0.408)	0.307 (0.202)	−0.137 (−0.234 to −0.041)	
72 h postoperatively	0.407 (0.349)	0.277 (0.219)	−0.136 (−0.233 to −0.038)	
Total 72 h AUC	36.307 (24.542)	27.004 (16.523)	−9.303 (−15.626 to −2.979)	0.004
CK (µg/L)				
Total AUC	32 542.8 (19 495.5)	36 312.3 (27 087.2)	3769.6 (−4647.0 to 12186.2)	0.377
AKI score (N)				
0	70 (79%)	80 (90%)		
1	11 (12%)	6 (7%)		
2	5 (6%)	2 (2%)		
3	3 (3%)	1 (1%)		
Total number of AKI cases	19 (21%)	9 (10%)		0.063
Inotrope score (mg/kg/h)				
Postbypass	6.8 (13.5)	6.8 (15.3)	0.0 (−4.8 to 4.8)	
24 h postoperatively	11.6 (20.9)	9.4 (16.6)	−2.2 (−7.0 to 2.6)	
48 h postoperatively	8.4 (19.1)	5.5 (14.1)	−2.9 (−7.7 to 2.0)	
72 h postoperatively	5.6 (16.8)	1.7 (8.3)	−3.9 (−8.7 to 1.0)	
Total	32.7 (58.8)	22.7 (42.3)	−10.0 (−25.6 to 5.6)	0.206
New onset AF (N)	22 (25%)	10 (11%)		0.031
Length of ICU stay (days)	3.0 (2.0–4.5)†	2 .0 (1.0–4.0)†		0.043‡
Length of hospital stay (days)	8.5 (7.0–12.0)†	8.0 (6.0–10.0)†		0.094‡
Clinical outcomes at 6 weeks (N)				
Death	5 (7%)	0 (0%)		0.057
Myocardial infarction	1 (1%)	0 (0%)		0.401
Stroke	0 (0%)	1 (1%)		0.451
Revascularisation	0 (0%)	0 (0%)		1.000

The following secondary end points had missing values: hsTnT 24 h postoperatively (RIPC 1), hsTnT 48 h postoperatively (control 1, RIPC 2), hsTnT 72 h postoperatively (control 3, RIPC 4), total 72 h AUC (control 3, RIPC 4), total AUC CK (control 29, RIPC 24), inotrope score postbypass (control 5, RIPC 4), inotrope score 24 h postoperatively (control 6, RIPC 4), inotrope score 48 h postoperatively (control 7, RIPC 4), inotrope score 72 h postoperatively (control 7, RIPC 4), total inotrope score (control 7, RIPC 4), length of ICU stay (control 1), length of hospital stay (control 1), death (control 15, RIPC 20), myocardial infarction (control 15, RIPC 20), stroke (control 15, RIPC 19), revascularisation (control 15, RIPC 20).
*Differences, 95% CIs of the differences and p value are calculated from repeated measures regression model.
†Results shown as median (IQR).
‡p Value for Mann–Whitney–Wilcoxon test.
AF, atrial fibrillation; AKI, acute kidney injury; AUC, area under the curve; CK, creatine kinase; IABP, intra-aortic balloon Pump; ICU, intensive care unit; RIPC, remote ischaemic preconditioning; hsTnT, high-sensitive troponin-T.

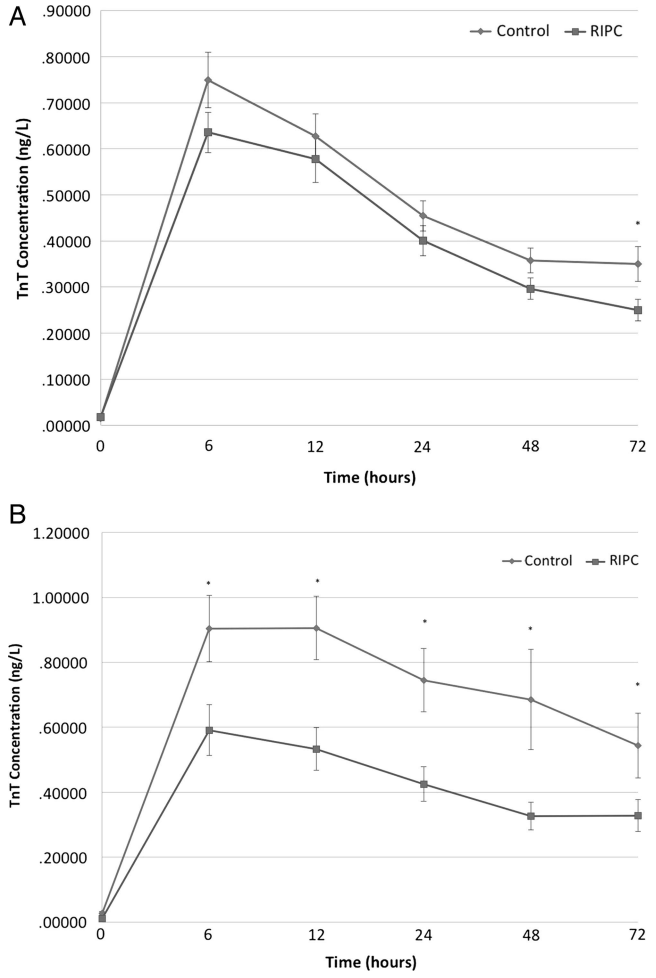

Figure 3 (A) Serum high-sensitivity troponin-T release in a subgroup of patients receiving intravenous GTN intraoperatively. Values are mean ±SEM. (B) Serum high-sensitivity troponin-T release in a subgroup of patients not receiving intravenous GTN intraoperatively. Values are mean±SEM. hsTnT, GTN, glyceryl trinitrate; high-sensitivity troponin-T; RIPC, remote ischaemic preconditioning.

requirement or major adverse cardiac events at 6 weeks in patients randomised to RIPC compared with control (table 3).

Post hoc subgroup analysis

We performed a post hoc subgroup analysis to examine the effect of administering an intravenous GTN infusion during surgery on the magnitude of PMI. Interestingly, we found that the total 72 h AUC hsTnT was reduced by 39% in those control patients who had been administered intraoperative intravenous GTN compared with those control patients who had not (GTN 30.8±17.6 µg/L vs no GTN 50.5±34.2 µg/L (−19.7 difference (CI −29.7 to −9.8); p<0.001; figure 3A; table 4), suggesting that intraoperative intravenous GTN itself can reduce PMI.

We also investigated whether administering an intravenous GTN infusion during surgery affected the cardioprotective efficacy of RIPC. RIPC did not reduce the magnitude of PMI in those patients who had been administered intraoperative intravenous GTN compared with those RIPC patients who had not (RIPC+GTN 26.7±13.9 µg/L vs RIPC+no GTN 27.9 ±20.10 µg/L (−1.2 difference (CI −9.9 to −7.6); p=0.793, figure 3B; table 4), suggesting that the beneficial effect of RIPC on PMI was absent in the presence of intraoperative intravenous GTN.

DISCUSSION

In an unselected prospective cohort of 180 adult patients undergoing elective CABG and/or valve surgery, we have demonstrated that a shortened RIPC protocol can reduce the amount of PMI by 26%. In addition, there is a possibility that RIPC may also improve short-term clinical outcomes with a 54% reduction in the incidence of postoperative AF, a 48% decrease in the incidence of AKI and a shortening of ICU stay by 1 day. However, the effect of RIPC on these outcome measures will have to be repeated in future studies.

A number of small clinical trials have investigated the effect of a standard single-limb RIPC protocol on the magnitude of PMI in patients undergoing cardiac surgery, the majority of which have demonstrated beneficial effects on PMI magnitude.[4 5 7] This has also been confirmed by a number of recent meta-analyses.[20] One clinical study has even suggested reduced mortality in preconditioned patients.[21] However, several recent studies have failed to demonstrate beneficial effects of RIPC in this patient group.[6 10 17] One potential explanation for these differences may relate to the RIPC stimulus itself, which may not be sufficient to elicit cardioprotection under certain conditions: the majority of clinical studies have used a standard single-limb RIPC protocol comprising either three or four 5 min cycles of inflation/deflation of a cuff placed on either the upper arm or thigh. In our study, we used a more intense RIPC protocol comprising two 5 min cycles of simultaneous upper arm and thigh cuff inflation and deflation, which can be delivered far more rapidly, requiring only 20 min, compared with 40 min using the standard single-limb four-cycle RIPC protocol. This allowed the multilimb RIPC protocol to be delivered after the induction of anaesthesia and well before sternotomy. Another potential explanation could be the timing of delivery of the RIPC stimulus: two of the negative studies that failed to report any beneficial effects with RIPC administered the protocol after sternotomy had taken place,[6 10] whereas in the vast majority of clinical studies the RIPC stimulus is initiated and completed prior to sternotomy.

A further possible reason for failing to observe RIPC cardioprotection in patients undergoing cardiac surgery may be due to concomitant therapy, including intravenous GTN: in a post hoc subgroup analysis of data, we investigated the effect of intraoperative intravenous GTN on PMI, in relation to the cardioprotective effect of RIPC. Interestingly, we found that control patients given intravenous GTN during surgery sustained 39% less PMI than control patients who did not receive intravenous GTN. Furthermore, in preconditioned patients administered intravenous GTN during surgery, RIPC had no beneficial effect on PMI, whereas in preconditioned patients not given intravenous GTN, RIPC significantly reduced the amount of PMI. It is well established in the published literature that nitric oxide donors such as GTN are highly effective mediators of cardioprotection in both the preclinical and the clinical settings.[22 23] These findings suggest that RIPC may not be able to elicit cardioprotection in the presence of intravenous GTN, as the myocardium may have already been protected by the GTN itself. In contrast to our findings, a recently published retrospective analysis by Kleinbongard *et al*[21] has suggested that intravenous GTN had no effect on RIPC cardioprotection in patients undergoing CABG surgery. Therefore, it will be important to investigate whether GTN is cardioprotective and whether RIPC cardioprotection is attenuated when it is present in a suitably powered prospective randomised controlled clinical trial.

In our study, the incidence of postoperative AF was reduced by 55% in RIPC-treated patients: new-onset AF occurs in 30%–50% of patients following cardiac surgery[24] and is associated

Table 4 Effect of intravenous glyceryl trinitrate (GTN) on plasma hsTnT levels in control and RIPC patients

hsTnT level (μg/L)	Control (mean(SD))	RIC (mean(SD))	Difference (95% CI)	p Value
Preoperatively				
GTN given	0.015 (0.015)	0.018 (0.023)	0.002 (−0.113 to 0.118)	
GTN not given	0.027 (0.027)	0.011 (0.015)	−0.016 (−0.188 to 0.156)	
6 h postoperatively				
GTN given	0.750 (0.487)	0.635 (0.319)	−0.114 (−0.229 to 0.001)	
GTN not given	0.904 (0.468)	0.591 (0.464)	−0.313 (−0.484 to −0.141)	
12 h postoperatively				
GTN given	0.627 (0.395)	0.578 (0.371)	−0.050 (−0.165 to 0.065)	
GTN not given	0.905 (0.447)	0.533 (0.389)	−0.372 (−0.544 to −0.200	
24 h postoperatively				
GTN given	0.454 (0.265)	0.401 (0.239)	−0.054 (−0.169 to 0.061)	
GTN not given	0.745 (0.446)	0.425 (0.313)	−0.324 (−0.497 to −0.152)	
48 h postoperatively				
GTN given	0.357 (0.214)	0.296 (0.168)	−0.065 (−0.181 to 0.050)	
GTN not given	0.701 (0.678)	0.327 (0.249)	−0.379 (−0.551 to −0.207)	
72 h postoperatively				
GTN given	0.350 (0.300)	0.250 (0.165)	−0.110 (−0.227 to −0.007)	
GTN not given	0.552 (0.435)	0.321 (0.281)	−0.235 (−0.408 to −0.063)	
72 h total AUC				
GTN given	30.811 (17.561)	26.687 (13.934)	−4.124 (−11.169 to 3.367)	0.006
GTN not given	50.515 (34.204)	27.858 (20.012)	−22.658 (−33.597 to −11.719)	

Differences, 95% CIs of the differences and p value for test of interaction between RIC and GTN given are calculated from a repeated measures regression model.
The following secondary end points had missing values: hsTnT 24 h postoperatively—GTN not given (RIPC 1), hsTnT 48 h postoperatively—GTN given (control 1, RIPC 1), hsTnT 48 h postoperatively—GTN not given (RIPC 1), hsTnT 72 h postoperatively—GTN given (control 3, RIPC 3), hsTnT 72 h postoperatively—GTN not given (RIPC 1), 72 h total AUC—GTN given (control 3, RIPC 3), 72 h total AUC—GTN not given (RIPC 1).
AUC, area under the curve; hsTnT, high-sensitivity troponin-T; RIC, remote ischaemic conditioning; RIPC, remote ischaemic preconditioning.

with increased rates of death, thromboembolic events, left ventricular failure, prolonged hospitalisation, reduced quality of life and poor exercise capacity.[24] The aetiology of postoperative AF is multifactorial, with acute myocardial IRI being one contributory factor.[24] Therefore, RIPC may have decreased the incidence of postoperative AF by protecting the myocardium against acute IRI. However, Rahman *et al*[6] failed to demonstrate any effect of RIPC on AF incidence following cardiac surgery. An ongoing large multicentre RICO trial is currently investigating the effects of RIPC on the incidence of postoperative AF in patients with CABG (ClinicalTrials.gov: NCT01107184).[25]

AKI can affect up to 30% of patients postcardiac surgery, necessitating dialysis in 1%–2% of cases and ultimately leading to an eightfold increase in death rate.[26] A number of clinical studies have investigated the effect of RIPC on postoperative renal function in patients undergoing cardiac surgery, where once again IRI plays a significant pathogenic role.[26] However, the results have been controversial:[6 10 11 27 28] our study is the largest to report a potential renoprotective effect with RIPC in patients undergoing cardiac surgery and found both an improved postoperative urine output and a reduced AKI incidence of 48% in preconditioned patients, although this did not reach statistical significance.

Finally, our study is the first to report beneficial effects of RIPC on the length of ICU stay following cardiac surgery: we found that RIPC shortened the duration of ICU stay by 1 day, a finding that may well be related to reduced PMI magnitude and decreased postoperative AF and AKI incidence.

One important limitation of our study was the blinding of the RIPC protocol in the anaesthetic room, which, because of the nature of the intervention, was difficult to achieve in an optimal manner. However, it is important to note that all data were collected by a research investigator blinded to the treatment allocation. Another limitation of our study was not adjusting for multiple comparisons and therefore, we restricted the number of comparisons we performed to the minimum in order to reduce the risk of a type I error. However, despite doing this, the effect of RIPC on clinical outcomes should be treated as 'hypothesis generating' and there is a possibility that the results may have arisen by chance, and therefore the clinical outcome data will need to be confirmed in future studies.

In summary, we have demonstrated that RIPC applied by simultaneous multilimb IRI can reduce the magnitude of PMI and

Key messages

What is known on this subject?
Remote ischaemic preconditioning (RIPC) may reduce perioperative myocardial injury in patients undergoing coronary artery bypass graft (CABG) surgery. Whether it can improve clinical outcomes in this patient group is unknown and is investigated in our study.

What might this study add?
We find that RIPC may improve short-term clinical outcomes as evidenced by reduced incidences of postoperative atrial fibrillation, acute kidney injury and a shortened ICU stay.

How might this impact on clinical practice?
RIPC may improve morbidity and mortality in patients undergoing CABG surgery and it, therefore, has the potential to change clinical practice.

has the potential to improve short-term clinical outcomes in an unselected cohort of adult patients undergoing elective CABG and/or valve surgery. However, the effect of RIPC on short-term clinical outcomes will have to be confirmed in future studies. Large multicentre randomised controlled clinical trials are currently being undertaken to evaluate the potential effects of RIPC on long-term clinical outcomes in patients undergoing CABG with or without valve surgery (ERICCA trial,[29] ClinicalTrial.gov identifier: NCT01247545 and RIPHeart trial,[30] ClinicalTrials.gov identifier: NCT01067703).

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Competing interests None.

Patient consent Obtained.

Ethics approval UCLH Ethics Committee.

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Heart

Effect of remote ischaemic preconditioning on clinical outcomes in patients undergoing cardiac bypass surgery: a randomised controlled clinical trial

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A retrospective analysis of Myocardial Preservation techniques during Coronary Artery Bypass Graft Surgery: Are we protecting the heart?

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Abstract

Background. Retrograde perfusion into coronary sinus during coronary artery bypass graft (CABG) surgery reduces the need for cardioplegic interruptions and ensures the distribution of cardioplegia to stenosed vessel territories, therefore enhancing the delivery of cardioplegia to the subendocardium. Peri-operative myocardial injury (PMI), as measured by the rise of serum level of cardiac biomarkers, has been associated with short and long-term clinical outcomes. We conducted a retrospective analysis to investigate whether the combination of antegrade and retrograde techniques of cardioplegia delivery is associated with a reduced PMI than that observed with the traditional methods of myocardial preservation.

Methods. Fifty-four consecutive patients underwent CABG surgery using either antegrade cold blood cardioplegia (group 1, n=28) or cross-clamp fibrillation (group 2, n=16) or antegrade retrograde warm blood cardioplegia (group 3, n=10). The study primary end-point was PMI, evaluated with total area under the curve (AUC) of high-sensitivity Troponin-T (hsTnT), measured pre-operatively and at 6, 12, 24, 48 and 72 hours post-surgery. Secondary endpoints were acute kidney injury (AKI) and inotrope scores, length of intensive care unit (ICU) and hospital stay, new onset atrial fibrillation (AF) and clinical outcomes at 6 weeks (death, non-fatal myocardial infarction, coronary artery revascularization, stroke).

Comparison between exposure groups was made by including the exposure variable as a categorical variable in a linear regression model for approximately normally distributed endpoint variables. For skewed endpoint variables the median T-test was used. For continuous endpoint variables measured over time a repeated measures linear regression model was fitted to measure the association between the exposure variable and endpoint. Categorical data were analysed using Fisher's exact test. No

adjustement for multiplicity has been made. Data were analysed using Stata version 12.1.

Results. There was evidence that mean total AUC of hsTnT was different among the three groups (P=0.050). In particular mean total AUC of hsTnT was significantly lower in group 3 compared to both group 1 (-16.55; 95% CI: -30.08, -3.01; P=0.018) with slightly weaker evidence of a lower mean hsTnT in group 3 when compared to group 2 (-15.13,; 95% CI -29.87, -0.39; P=0.044). There was no evidence of a difference when comparing group 2 to group 1 (-1.42,; 95% CI: -12.95, 10.12, P=0.806).

Conclusions. Our retrospective analayis suggests that, compared to traditional methods of myocardial preservation, antegrade retrograde cardioplegia may reduce PMI in patients undergoing first time CABG surgery.

Key Words

Coronary Artery Bypass Graft Surgery, Peri-operative Myocardial Injury, Antegrade Cardioplegia, Retrograde Cardioplegia, Cross-clamp Fibrillation

Background

The prompt delivery of cardioplegic solution to all regions of the heart during cardiac surgery is essential for successful myocardial protection[1]. PMI, detected through serial measurements of specific serum biomarkers including troponin I (TnI) or T (TnT) or creatine kinase (CK)-MB, is associated with worse clinical outcomes[2-7].

Antegrade delivery of cardioplegic solution to myocardial cells is adequate when supplied by unobstructed coronary arteries but may not be equally effective in the presence of occluded or stenosed arteries as in the case of coronary artery disease (CAD), which may lead to maldistribution of cardioplegic solution[8]. This might induce PMI and poor recovery of left ventricular function (LV) following revascularization[9]. Limitations associated with antegrade delivery may be circumvented by the administration of cardioplegic solution in a retrograde fashion through the coronary sinus which relies on the principle that coronary venous systems free of disease and valves can serve as a conduit for the delivery of cardioplegic solution in a homogenous manner[10). With this technique, the cardioplegic solution is distributed to the cardiac microstructure through a transmural network of veins that is independent to flow-limiting lesions[11]. Nevertheless, retrograde cardioplegia presents important potential limitations, which could in part explain the reason why its use remains still relatively limited:

1) the anterior cardiac veins supplying the right ventricle (RV) are not directly connected to the coronary sinus and this may lead to a suboptimal distribution of the cardioplegic solution to the RV[12,13];
2) accurate cannulation of the coronary sinus is crucial as failure in this might lead to the distribution of the cardioplegic solution to the right atrium and not to the venous system;

3) the perfusion pressure requires very close monitoring, as too low a pressure suggests misplacement of the cannula, and too high a pressure can cause rupture of the coronary sinus[14,15]. These potential issues can generally be avoided by care and precision by the surgeon;
4) the delay in arresting the heart due to slow retrograde perfusion if retrograde cardioplegia is used alone (lower flow rates and pressures used to prevent coronary sinus damage and myocardial oedema)[16,17].

Therefore, the combination of both methods of antegrade and retrograde cardioplegia is thought to overcome limitations inherent to both techniques[18]. However, although both antegrade perfusion and retrograde coronary sinus perfusion have been studied experimentally and used clinically in patients undergoing CABG surgery, there is little information comparing PMI magnitude between different methods of cardioprotection during revascularization. In this regards, we conducted a retrospective subgroup analysis to determine whether the combination of antegrade and retrograde cardioplegia is associated with improved myocardial preservation in patients undergoing CABG surgery.

Methods

Study design

We conducted a retrospective analysis of patients undergoing first time CABG surgery recruited between December 2010 and July 2012 as a subgroup of control patients in a large parallel single-blinded randomised controlled clinical trial carried out at the Heart Hospital, University College London Hospital (London, UK), and investigating the effects of remote ischaemic preconditioning (RIPC)[4,5] in patients

undergoing cardiac surgery. The surgery was carried out in accordance with the University College London Hospital NHS Trust guidelines and received local Ethics Committee approval. We obtained written informed consent from all patients recruited into the study.

Inclusion and Exclusion Criteria

Patients included in the current analysis were recruited within a single centre study investigating the effects of RIPC[19,20] in patients undergoing CABG surgery at the Heart Hospital (UCLH London, UK): only patients receiving the control protocol were included.

Patient exclusion criteria comprised:

a) cardiogenic shock or cardiac arrest preceding surgery;
b) positive baseline serum hsTnT;
c) pregnancy;
d) significant peripheral arterial disease (PAD) affecting upper and/or lower limbs;
e) significant hepatic dysfunction (International Normalised Ratio>2.0);
f) significant pulmonary disease (Forced Expiratory Volume-1<40% predicted);
g) renal failure with an estimated glomerular filtration rate <30 mL/min/1.73 m^2;
h) concomitant therapy with glibenclamide or nicorandil, as these medications have been demonstrated to potentially interfere with RIPC.

Surgical procedure

Temazepam 10-20 mg was given one hour prior to surgery. Anaesthesia induction was obtained under cardiac monitoring with combinations of midazolam, etomidate, propofol, fentanyl and anti-nicotinic agents (rocuronium, vecuronium or pancuronium). Endotracheal intubation was achieved and mechanical ventilation commenced with oxygen with or without air. For anaesthesia maintenance volatile anaesthetic agents and propofol infusion with or without fentanyl were used. Continuous monitoring of arterial blood pressure, central venous pressure, nasopharyngeal temperature was carried out.

Standard non-pulsatile cardiopulmonary bypass (CPB) was initiated with the use of membrane oxygenator and cardiotomy suction, followed by the construction of all coronary artery bypass grafts, using either blood cardioplegia or intermittent cross-clamp fibrillation.

Group 1 had 1 litre of cold blood cardioplegia given once the cross clamp was placed and maintenance cold blood cardioplegia was given down the grafts in occluded arteries and also into the aortic root every 20-30 minutes.

Group 2 had coronary artery surgery with the cross clamp fibrillation technique and therefore no cardioplegic solution was given.

Group 3 had warm blood cardioplegia delivered with antegrade and retrograde techniques and was performed by one cardiothoracic surgeon, with an initial 800ml dose of antegrade cardioplegia followed by 400ml of retrograde cardioplegia. After this, maintenance was achieved with 100ml of retrograde cardioplegia after each anastamosis. A hot shot of warm blood without potassium was given after the internal mammary artery (IMA) anastamosis and prior to removal of the cross clamp. All

anastomoses were constructed with the single clamp technique. Systemic temperature in group 3 was 35°C.

Following anastomosis of the grafts, CPB was discontinued and protamine was used to achieve heparin reversal.

Objectives

To determine whether the addition of retrograde cardioplegia to standard antegrade cardioplegia can reduce PMI and subsequently improve short-term clinical outcomes in patients undergoing first time CABG surgery compared to patients receiving either standard antegrade cardioplegia alone or cross-clamp fibrillation.

Study Endpoints

The primary study end-point was PMI, measured by total 72 hour AUC of hsTnT. Blood samples for hsTnT were taken pre-operatively and at 6, 12, 24, 48 and 72 hours post-surgery; hsTnT was measured quantitatively by a one-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche, Switzerland). The lower detection limit of this assay is 1ng/L with a threshold of ≥14 ng/L indicating significant myocardial necrosis.

Secondary end-points included:

1. AKI score, classified as grade 1, 2 or 3 based on a combination of laboratory (serum creatinine levels) and clinical (urine output) parameters[21]. Serum creatinine was measured pre-operatively and 24, 48 and 72 hours post-surgery.

2. Inotrope requirement during the first 3 post-operative days, measured using the formula modified from a study by Ko et al.[22]:

   Inotrope score= Dosages (μg/kg/min) of:

   [Dopamine + Dobutamine + Dopeximine] +

   [(Adrenaline+ Noradrenaline + Isoprotenerol) x 100] + [(Enoximone + Milrinone) x 15];

3.Length of ICU and hospital stay;

4. New onset of AF in the three post-operative days;

5. Clinical outcomes at 6 weeks (death, non-fatal myocardial infarction, coronary artery revascularization, stroke).

Statistical analysis

Continouos data are presented as mean (standard deviation (SD)) or median (IQR). Categorical data are shown as number (percent). The exposure variable was a categorical variable with three groups corresponding to each of the cardioprotection categories. Comparison between exposure groups was made by including the exposure variable as a categorical variable in a linear regression model for approximately normally distributed endpoint variables. For very skewed endpoint variables the median T-test was used. Where continuous endpoint variables were measured over time a repeated measures linear regression model was fitted to measure the association between exposure variable and endpoint. The assumptions of the linear regression models were performed by analysis of residuals. Categorical data were analysed using Fisher's exact test. No adjustment for multiplicity has been made. Data were analysed using Stata version 12.1.

Results

Included patients were recruited into an original RIPC trial enrolling a total of 180 subjects of which 90 patients were randomised to control: 36 patients were subsequently excluded (1 patient died intra-operatively and the remaining 35 underwent CABG and valve surgery or valve surgery alone). Therefore we ultimately analysed data for 54 patients undergoing CABG surgery alone: 28 received antegrade cold blood cardioplegia (group 1), 16 patients received cross-clamp fibrillation (group 2) and 10 antegrade retrograde warm blood cardioplegia (group 3).

Group 3 had a lower rate of positive family history of CAD and previous percutaneous coronary intervention (p=0.047) whereas group 3 had a higher incidence of cerebro-vascular accidents prior to CABG surgery (p=0.025, **Table 1**): no other significant difference was found among the three groups with respect to baseline patient baseline characteristics (**Table 1**). Expectedly, cross-clamp time was significantly lower in group 2 (p<0.001), however all the remaining parameters of surgery were similar amongst the 3 groups (**Table 1**).

Primary endpoint

Overall there was evidence that the mean total 72 hr AUC hsTnT was different among the three groups (P=0.050). Examining the subgroup differences showed evidence of lower mean hsTnT in group 3 compared to group 1 (-16.55; 95% CI: -30.08, -3.01; P=0.018) with slightly weaker evidence of a lower mean hsTnT in group 3 when compared to group 2 (-15.13,; 95% CI -29.87, -0.39; P=0.044). There was no evidence of a difference when comparing group 2 to group 1 (-1.42,; 95% CI: -12.95, 10.12, P=0.806) (**Figure 1, Table 2**).

Secondary endpoints

Baseline pre-operative hsTnT levels were <0.02 μg/L and not significantly different between the 3 groups (**Figure 2, Table 2**). Overall there was evidence that the mean hsTnT differed at 6 hours (P<0.001) and 12 hours (P<0.001). At 6 hours there was evidence that mean hsTnT was lower in group 3 than groups 1 (-0.56; 95% CI: -0.78, -0.34). Similarly at 12 hours there was some evidence that mean hsTnT was lower in group 3 than group 1 (-0.43, 95% CI: -0.65, -0.21) (**Figure 2, Table 2**).

There was no evidence of a significant difference among the three groups with regards to each of the secondary endpoints **(Table 3).**

Discussion

Myocardial preservation during cardiac surgery is certainly one of the most debated topics in this field. One method of achieving myocardial protection is by using a cardioplegic solution administered into the heart to achieve a temporary arrest of the myocardium whilst the surgeon performs the operation in a bloodless field.

In the UK a variety of myocardial protection strategies are used, including cold blood antegrade cardioplegia with topical cooling, warm blood antegrade cardioplegia, warm blood antegrade and retrograde cardioplegia, cold blood antegrade and retrograde cardioplegia and cross clamp fibrillation.

In current practice the route of delivery is at the surgeon's discretion and as such there is no consensus on using a specific route to supply the cardioplegia into the myocardium.

The most common technique used by the majority of cardiac surgeons is the antegrade route, in which cardioplegia is delivered into the aortic root and spreads via

the coronary arteries supplying the myocardium. Although significant clinical evidence favours the safety of this method, severe stenoses of coronary arteries in patients undergoing CABG may prevent the uniform distribution of cardioplegic solution through the myocardium[23] and, importantly, sub-optimal or inadequate distribution to parts of the myocardium increases the risk for PMI.

A proposed solution to overcome this limitation is the retrograde route of delivery, in which cardioplegia is administered through the coronary sinus and through the extensive venous network of the myocardium. Following the pioneering idea of Pratts[24] who suggested that an ischemic myocardium could be revived back into its healthy form by supplying oxygenated blood through the veins, retrograde cardioplegia was applied for the first time by Blanco et al[25] in 1956 and further developed in a significant number of centres: one of the seminal works on retrograde cardioplegia was conducted by Menasche et al.[26] who demonstrated that, during aortic valve replacement (AVR) surgery, post-operative haemodynamic stability, cardiac outputs and right and left ventricular stroke indices were similar in patients receiving retrograde cardioplegia compared to those receiving antegrade cardioplegia. The same authors also conducted a retrospective observational study on a relatively large group of patients undergoing isolated AVR or CABG surgery with or without concomitant valve surgery using retrograde cardioplegia alone[27] and, although there was no antegrade cardioplegia group for direct comparison, they reported that the overall trend in mortality rates was either similar or less than what other studies had shown.

With the knowledge that the anterior cardiac veins supplying the RV are not directly connected to the coronary sinus and thus may lead to a suboptimal distribution of the cardioplegic solution to the RV[12,13] Kaukoranta et al.[28] conducted

a small study on patients undergoing CABG surgery and receiving either antegrade or retrograde cardioplegia and reported that, despite more significant ischaemic changes within the RV in the retrograde cardioplegia group, no post-operative complication related to the retrograde route was observed.

It is therefore clear from the literature that multifactorial clinical endpoints have been used to determine a difference between myocardial protection strategies: moreover the population size in these studies was often too small to come to a meaningful conclusion on the benefit of a particular protection strategy. Importantly, many of the studies on retrograde cardioplegia used retrograde cardioplegia alone when compared to antegrade cardioplegia and only very few studies have compared the combination of antegrade and retrograde techniques against antegrade cardioplegia alone: this is one more reason to interpret current literature with caution.

In the only other study similar to our retrospective analysis, Radmehr and his colleagues[13] compared the combined antegrade and retrograde versus antegrade cardioplegia alone in patients undergoing CABG surgery and found that the use of combined antegrade and retrograde cardioplegia was associated with a 16.5% statistically significant decrease in inotropic requirement.

Our retrospective study suggests that myocardial protection can be improved by combined antegrade and retrograde technique and, in contrast to literature already available, our patient cohort is divided into three groups (the combined technique of antegrade and retrograde cardioplegia, antegrade cardioplegia alone and cross-clamp fibrillation). We only included patients undergoing CABG surgery and excluded subjects undergoing either valve surgery alone or CABG combined with valve surgery. This enabled us to assess the drawbacks of the antegrade technique via blocked coronaries and therefore the potential benefit of retrograde cardioplegia.

In addition to this, with an increase in the cross-clamp time the general understanding is that the myocardium is more prone to becoming ischaemic and damaged: our study suggests that despite longer cross-clamping times in the combined group, the total PMI was consistently lower in these patients when compared to the other two groups. This correlates with a retrospective study conducted by Bar-El et al.[29] on patients undergoing CABG surgery with or without valve repair or replacement and receiving antegrade followed by retrograde cardioplegia, demonstrating that the expected mortality was lower in patients with longer aortic cross-clamping times compared to those with shorter aortic cross-clamping times, and therefore indicating that retrograde cardioplegia can enhance myocardial protection despite the longer cross-clamp times. We therefore suggest that the absolute value of the cross-clamp time may potentially be less important than the type of myocardial protection used: this could be crucial in complex patients with poor LV function or anticipated long cross-clamp times, for whom the best myocardial protection available would be warranted.

Another important difference between groups 1 and 3 is the temperature employed during the cardioplegic techniques utilizing cold blood and warm blood cardioplegia respectively: the optimal temperature of cardioplegia during cardiac surgery is another crucial aspect of myocardial protection besides the actual technique used and it could be argued that the lower troponin rise in group 3 may be partially explained by the temperature difference. Cold cardioplegia is able to attenuate myocardial oxygen demand and the risk of ischaemic damage but conversely may lead to the inhibition of myocardial enzymes leading to a stunning of the metabolic and functional recovery following surgery. However warm blood cardioplegia is thought to counteract this potential deleterious effect. In a meta-analysis[30] involving

8814 patients randomised to either warm cardioplegia or cold cardioplegia predominantly in the setting of CABG surgery, there was no significant difference in all-cause mortality or incidence of myocardial infarction, intra-aortic balloon pump usage, stroke, low output syndromes and post-operative AF between the two patients groups and postoperative cardiac index was significantly improved in the warm blood cardioplegia group. Similarly, Tan *et al.*[31] compared cold to tepid cardioplegia and found no difference in mortality, peri-operative myocardial infarction, stroke or inotrope requirement.

Our retrospective study has several limitations. The cohort population was small and in particular the group consisting of the combined use of retrograde cardioplegia and antegrade cardioplegia contained only 10 patients, who were operated on by one consultant, which may result in some potential bias. Typically in a study of this type strong prognostic and confounding variables would be adjusted for in the analysis. In this case the small sample size precluded detailed adjustment and we acknowledge some residual confounding bias may remain. Finally, we have not adjusted for multiplicity in our analysis and there is a possibility that the results may have arisen by chance, and therefore the clinical outcome data will need to be confirmed in future studies.

In conclusion our retrospective clinical analysis suggests that the combined use of retrograde cardioplegia and antegrade cardioplegia during first time CABG surgery can be beneficial in reducing PMI. Also, importantly, to our knowledge there is no study which combines the following four factors together into one analysis on PMI: aortic cross-clamping times, combined antegrade retrograde versus antegrade alone versus no cardioplegia, hsTnT levels at 6 different time-points with AUC-

hsTnT, and exclusively CABG patients. We do not suggest surgeons to change their practice for routine CABG surgery, as we have not yet demonstrated that the change in measured troponin levels may have a significant impact on clinical outcomes. However, we feel that this evidence should be available, so surgeons can choose to add retrograde cardioplegia for more complex cases in an evidence based fashion, knowing that the addition of retrograde cardioplegia may have the potential to enhance myocardial protection. Our retrospective study also suggests that larger studies are required in order to further evaluate our findings and to investigate whether the reduction of PMI in patients undergoing CABG would result in improvement of clinical outcomes.

Conclusions

Our study suggests that, compared to traditional methods of myocardial preservation, the combined use of retrograde and antegrade cardioplegia may have the potential to reduce PMI in patients undergoing first-time CABG surgery. Whether this can lead to an improvement of clinical outcomes is yet unknown and therefore larger studies are required in order to further evaluate this potential impact.

List of Abbreviations

CABG= coronary artery bypass graft

PMI= peri-operative myocardial injury

AUC= area under the curve

hsTnT= high-sensitivity Troponin-T

AKI= acute kidney injury

ICU= intensive care unit

AF= atrial fibrillation

SD= standard deviation

CK= creatine kinase

CAD= coronary artery disease

RV= right ventricle

RIPC= remote ischaemic preconditioning

PAD= peripheral arterial disease

CPB= cardiopulmonary bypass

Competing interests

There are no finanicial and non-financial competing interests.

Authors' contributions

LC and AM carried out the study, acquired, analysed and interpreted data.

LC, AM. SAK, NR made substantial contributions in drafting the manuscript and/or revising it critically for important intellectual content.

CA performed the statistical analysis

NR, MB, CDS, DRL, MPH, JAP, AMS, SKK provided patients for the study

DJH, NR read and approved final manuscript

All authors read and approved the final manuscript

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Figures legends

Figure 1. Mean hsTnT pre-operatively and at 6, 12, 24, 48 and 72 hours post-surgery in the three study groups.

Figure 2. AUC of hsTnT over the 72 hours post-surgery in the three study groups.

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Figures

Fig. 1

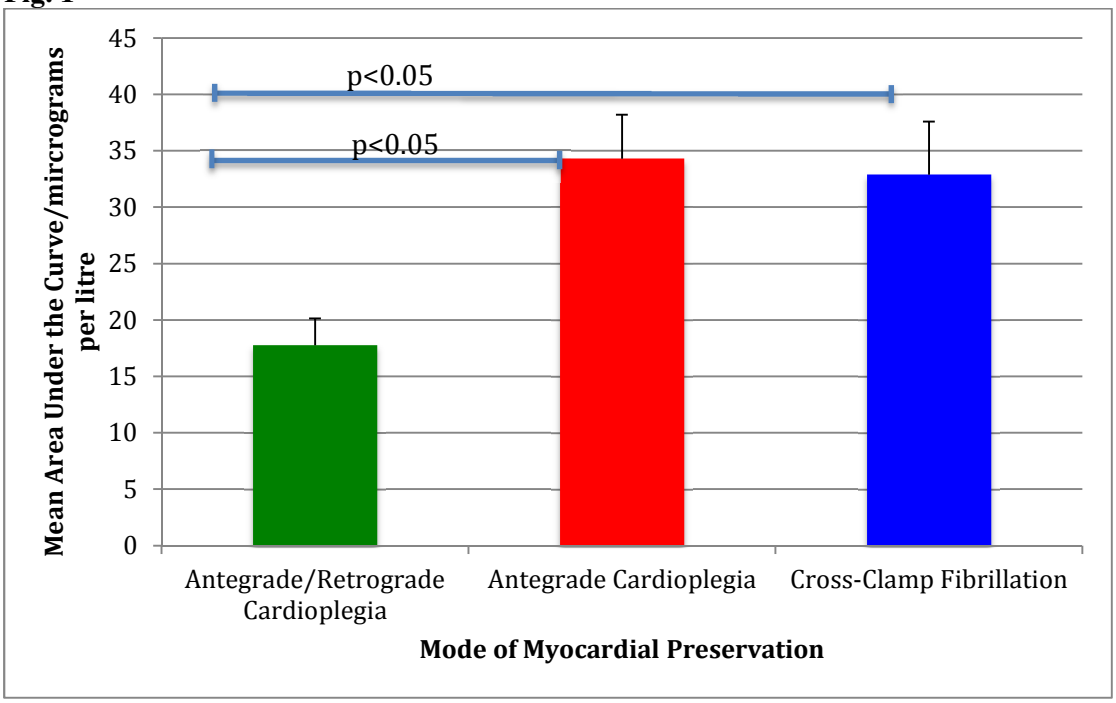

Figure 1

Figures

Fig. 2

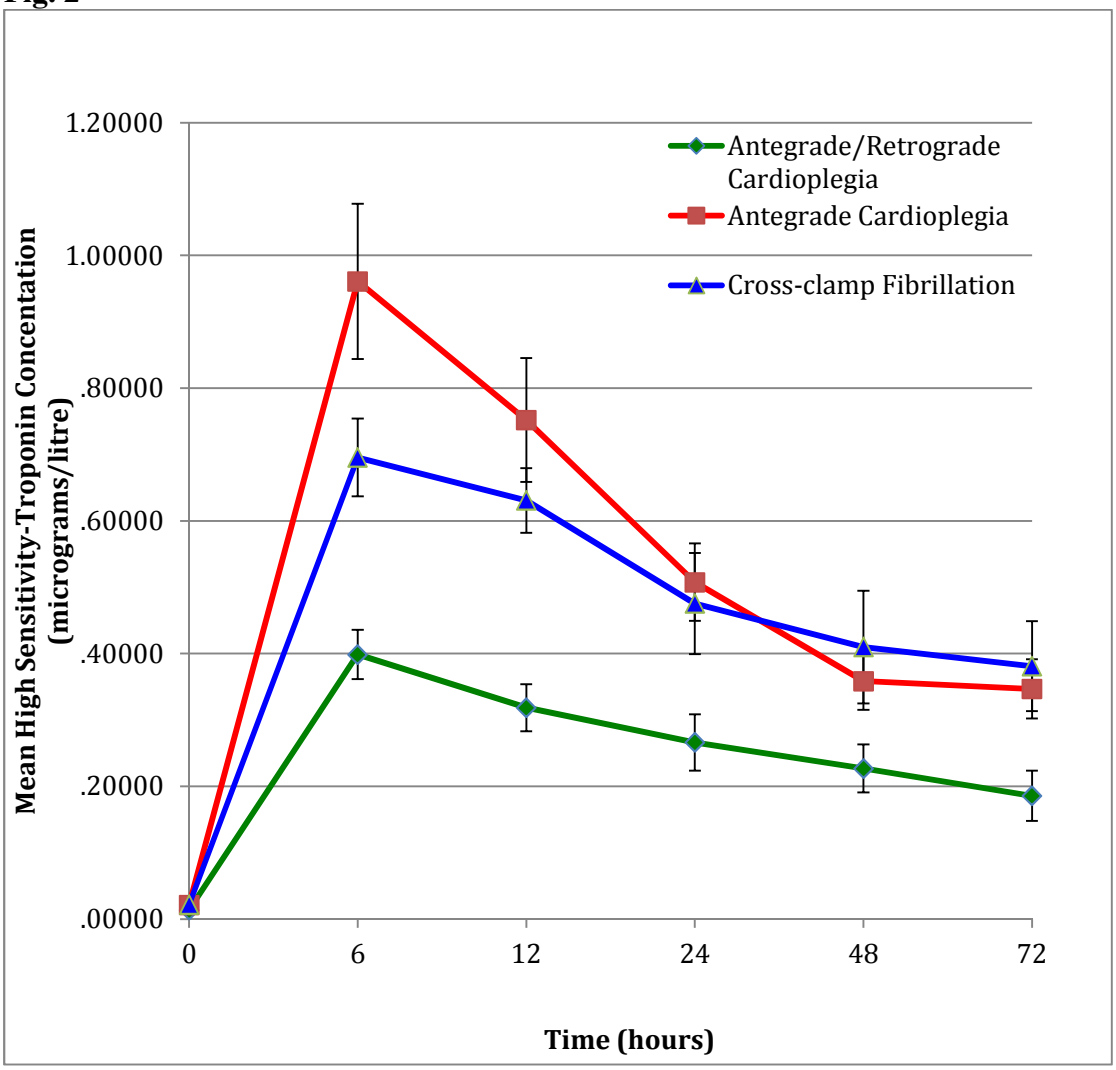

Figure 2

Additional files provided with this submission:

Additional file 1: 19092014_Table_1_.docx, 24K
http://www.cardiothoracicsurgery.org/imedia/1753576742143271/supp1.docx
Additional file 2: 19092014_Table_2[1].docx, 21K
http://www.cardiothoracicsurgery.org/imedia/1289561656143271/supp2.docx
Additional file 3: 19092014_Table_3[1].docx, 19K
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ORIGINAL PAPER

Effect of remote ischemic preconditioning on clinical outcomes in patients undergoing coronary artery bypass graft surgery (ERICCA): rationale and study design of a multi-centre randomized double-blinded controlled clinical trial

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Abstract
Background Novel cardioprotective strategies are required to improve clinical outcomes in high risk patients undergoing coronary artery bypass graft (CABG) ± valve surgery. Remote ischemic preconditioning (RIC), in which brief episodes of non-lethal ischemia and reperfusion are applied to the arm or leg, has been demonstrated to reduce perioperative myocardial injury following CABG ± valve surgery. Whether RIC can improve clinical outcomes in this setting is unknown and is investigated in the effect of remote ischemic preconditioning on clinical outcomes (ERICCA) trial in patients undergoing CABG surgery. (ClinicalTrials.gov Identifier: NCT01247545).
Methods The ERICCA trial is a multicentre randomized double-blinded controlled clinical trial which will recruit 1,610 high-risk patients (Additive Euroscore ≥ 5) undergoing CABG ± valve surgery using blood cardioplegia via 27 tertiary centres over 2 years. The primary combined endpoint will be cardiovascular death, non-fatal myocardial infarction, coronary revascularization and stroke at 1 year. Secondary endpoints will include peri-operative myocardial and acute kidney injury, intensive care unit and hospital stay, inotrope score, left ventricular ejection fraction, changes of quality of life and exercise tolerance. Patients will be randomized to receive after induction of anesthesia either RIC (4 cycles of 5 min inflation to 200 mmHg and 5 min deflation of a blood pressure cuff placed on the upper arm) or sham RIC (4 cycles of simulated inflations and deflations of the blood pressure cuff).
Implications The findings from the ERICCA trial have the potential to demonstrate that RIC, a simple, non-invasive and virtually cost-free intervention, can improve clinical outcomes in higher-risk patients undergoing CABG ± valve surgery.

Keywords Remote preconditioning · Ischaemia · Reperfusion · Clinical trial · CABG surgery

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Background

Coronary heart disease (CHD) is the leading cause of death and disability worldwide and accounts for 3.8 million of men and 3.4 million of women deaths every year. CABG surgery remains the procedure of choice for coronary artery revascularization in patients with multi-vessel coronary artery disease. Currently, high-risk CHD patients are being operated on due to the aging population, the increasing prevalence of co-morbidities such as diabetes, hypertension, valve disease and the presence of more complex

disease [1]. Surgery in these patients is associated with an elevated overall operative risk of 5–6% compared to 1% in lower-risk patients [2], as well as an increased risk of peri-procedural myocardial injury (as measured by serum CK-MB, Troponin T or I) [3], acute kidney injury (AKI) [4] and stroke [5], the presence of which are associated with worse clinical outcomes [6–16]. Myocardial injury during CABG surgery may be attributed to different mechanisms, including most importantly acute myocardial ischemia–reperfusion injury (IRI), but also inflammatory response to the extraneous substances in the cardiopulmonary bypass circuit, left ventricular over-distension, coronary athero-embolism [17], increased cardiac workload during the intraoperative period, and direct myocardial injury due to retraction and handling of the heart [18].

According to the technique used, myocardial IRI can be the result of intermittent cross-clamping, intermittent or continuous administration of cardioplegic solution, cross-clamp fibrillation or a combination of these methods and may manifest as myocardial stunning [19], the so called no-reflow phenomenon [20], reperfusion arrhythmias [21] and lethal reperfusion injury [22], the latter of which would be of most concern. A variety of factors are believed to contribute to lethal myocardial IRI and include oxidative stress [23], pH changes [24] calcium overload [22], the acute inflammatory response [25] and important metabolic changes [26], many of which impact on the opening of the mitochondrial permeability transition pore (mPTP), a critical determinant of cell death in the setting of acute IRI [27].

Whilst cardioprotective strategies have been overall extremely encouraging in experimental studies, the translation from bench to bedside has not always resulted in positive outcomes and this could be due to the obvious differences between the species involved, the size and age of animals used and the absence of co-morbidities and concomitant treatments in the experimental models [28, 29].

Both pharmacological and non-pharmacological interventions have been investigated to enhance the innate process of cardioprotection and therefore to limit or prevent acute myocardial IRI. Amongst the non-pharmacological strategies, ischemic preconditioning (IPC), per-conditioning (IPerC) and postconditioning (IPost) have been extensively investigated in both the laboratory and clinical settings. In IPC, the heart is subject to brief episodes of non-lethal ischemia and reperfusion prior to the sustained episode of lethal ischemia and reperfusion; in IPerC and IPost the protective stimulus is applied after the onset of myocardial ischemia or at the time of myocardial reperfusion, respectively [30]. However, both IPerC and IPost require an invasive intervention applied directly to the myocardium in order to achieve cardioprotection and may therefore be impractical or even harmful, particularly in the setting of an acute myocardial infarction (AMI). In this perspective, the phenomenon of remote ischemic conditioning (RIC) appears extremely encouraging: it describes the phenomenon in which brief episodes of non-lethal ischemia and reperfusion to an organ (i.e. kidney, liver or small intestine) or tissue (i.e. skeletal muscle), protect the heart against a sustained episode of lethal IRI [31, 32]. Therefore, RIC does not imply a direct intervention on the heart to achieve cardioprotection.

The concept of RIC was first introduced in 1993 by Przyklenk et al. [31], who demonstrated that IPC protects canine myocardium both in the territory exposed to brief coronary occlusion and in a vascular bed distant or remote or "virgin". Following this, pioneering studies by MacAllister et al. [33] demonstrated that RIC could be reproduced by non-invasively applying brief episodes of ischemia and reperfusion to the forearm using a standard blood pressure cuff. Since then, a number of proof-of-concept clinical studies have demonstrated that RIC comprising brief episodes of non-lethal ischemia and reperfusion to the arm or leg, non-invasively applied by inflating a blood pressure to supra-systolic pressures placed on the arm or leg, can protect the heart during CABG surgery from peri-operative myocardial injury as evidenced by reduced serum troponin T [34–37]. A similar RIC stimulus has been reported to be protective in a number of different clinical settings including elective surgical repair of abdominal aortic aneurysm (AAA) [38, 39], elective cervical decompression surgery [40]; elective PCI [41] and in ST-segment elevation myocardial infarction patients undergoing primary percutaneous intervention (PCI) [42].

Currently, whether this non-invasive virtually cost-free intervention can improve clinical outcomes in higher-risk patients undergoing CABG ± valve surgery is unknown and is the objective of the proposed effect of remote ischemic preconditioning on clinical outcomes (ERICCA) trial in patients undergoing coronary artery bypass graft (CABG) surgery. (ClinicalTrials.gov Identifier: NCT01247545).

Methods

Study objectives

The primary objective of this study is to determine whether RIC improves 1 year clinical outcomes (cardiovascular death, non-fatal myocardial infarction, revascularization and stroke) in higher-risk adult patients undergoing CABG ± valve surgery. The secondary research objectives are to determine whether RIC improves the above clinical outcomes at 30 days post-surgery; has an effect on all-cause death; reduces peri-operative myocardial injury and preserves LV systolic function; reduces AKI; improves

patient morbidity [intensive care unit (ICU) stay and hospital stay], lessens inotrope requirements and improves exercise tolerance and quality of life.

Study design

The study has received Ethical Committee approval. The ERICCA trial is a controlled randomized multi-centre double blind trial. It investigates the effect of RIC on 1 year clinical outcomes in 1,610 high-risk (Euroscore ≥ 5) patients undergoing CABG $\pm$ valve surgery recruited via 27 tertiary cardiac centres in the UK.

Study population

Patient inclusion criteria are as follows: the patient is $\geq$18 years old, scheduled for CABG $\pm$ valve surgery with blood cardioplegia, and has an additive Euroscore equal or above 5 (Fig. 1). Patient exclusion criteria include the following: history of cardiogenic shock or cardiac arrest during the current admission; pregnancy; significant peripheral arterial disease affecting the upper limbs; significant hepatic impairment (Bilirubin $>$ 20 mmol/L, INR $>$ 2.0); significant pulmonary disease (FEV1 $<$ 40% predicted); renal failure with a GFR $<$30 mL/min/1.73 m^2; concomitant treatment with glibenclamide or nicorandil (as these medications may interfere with the cardioprotection elicited by RIC). All patients will freely give their informed consent to participate in the study and may decide to withdraw from the study at any time. The study will conform to the spirit and the letter of the declaration of Helsinki, and in accordance with the UCL Good Clinical Practice Guidelines.

Intervention

The intervention being assessed is RIC, which will be performed after the induction of anesthesia but prior to surgery and will occur within 1 hour of the institution of cardiac bypass. Those patients randomized to receive RIC will have a standard blood pressure cuff placed on the upper arm, inflated to 200 mmHg for 5 min and then deflated for 5 min, a cycle which will be performed four times in total. For patients with systolic blood pressures $>$185 mmHg, the cuff will be inflated to at least 15 mmHg above the patient's systolic blood pressure. The sham RIC protocol is described as follows: the air valve on the blood pressure cuff is first opened such that the cuff is not inflated on squeezing the attached bulb. The bulb will then be squeezed for a duration of 15 s to give the impression that the cuff is being inflated. After 5 min, the air valve will be closed to give the impression that the cuff is being deflated. After 5 min, the air valve will be opened again and the bulb squeezed as before: the above cycle will be repeated four times in total. This is to ensure the rigorous blindness of the anesthetic and surgical teams as well as non-medical theatre staff. These interventions will be undertaken after the induction of anesthesia and will not prolong the anesthetic time or delay the onset of surgery.

We have decided to use four cycles of 5 min of cuff inflation and deflation as we wish to maximize the RIC stimulus to overcome potential resistance of diabetic heart and the presence of other factors interfering with cardioprotection such as volatile anesthetics including isoflurane. Moreover, the application of four cycles of 5 min inflation/deflation was reported to be beneficial in patients with STEMI undergoing primary PCI [42].

Randomization and allocation

On the morning of surgery, patients will be randomized to one of two groups, either RIC or control. Randomization will be coordinated centrally by the Clinical Trials Unit based at the London School of Hygiene and Tropical Medicine via a secure web-site and will be stratified by center using random permuted blocks. This will only be accessed by the research nurse responsible for performing either the RIC or sham RIC protocol. The same research nurse will be the only person in each centre aware of the treatment allocation for the patient and he/she will not be involved with the data collection other than those relating to the actual randomization procedure. Treatment allocations will only be known by one research nurse at each centre. The patient, cardiac surgeons and anesthetists, the research nurses collecting the data, and the assessor of clinical outcomes will all be blinded to the treatment allocation.

Study endpoints

Primary clinical endpoint

Major adverse cardiac events (cardiovascular death, non-fatal myocardial infarction, repeat revascularization) and cerebrovascular events (stroke) calculated at 12 months post-surgery.

Cardiovascular death will be defined as death due to a known cardiovascular cause or where the cause of death is unknown, i.e. where no other cause of death has been identified from the medical history or an autopsy.

Repeat revascularization will be defined as any PCI or repeat-CABG $\pm$ valve surgery within the first year post-surgery.

Myocardial infarction will include both peri-operative myocardial infarction and myocardial infarction following

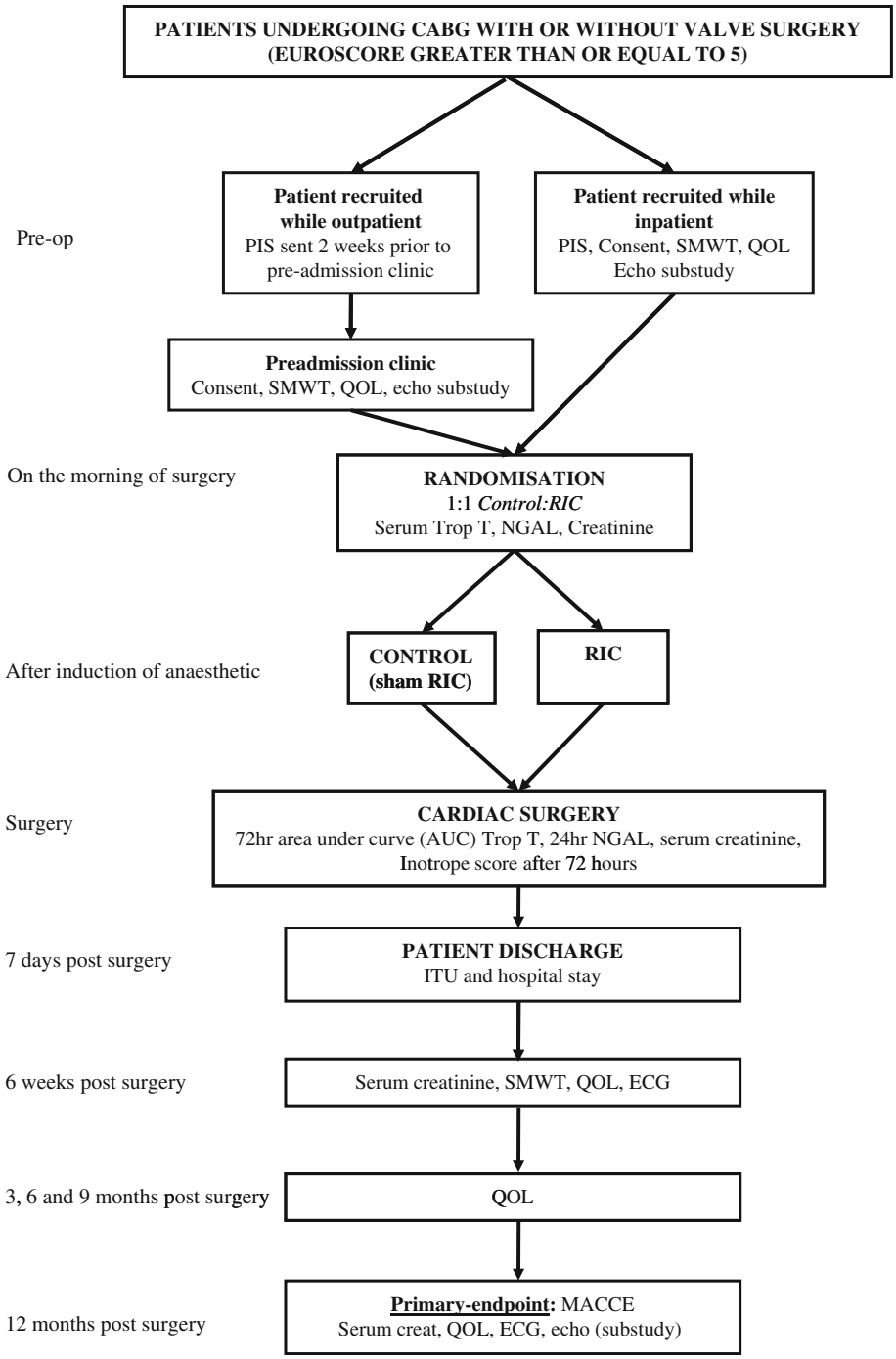

Fig. 1 Study flow chart

cardiac surgery. Peri-operative myocardial infarction (type 5 myocardial infarction) [43] will be indicated by biomarker (high-sensitive Troponin T) values more than five times the 99th percentile of the normal reference range during the first 72 h following CABG ± valve surgery, when associated with the appearance of new pathological Q-waves or new left bundle branch block (LBBB), or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium. Post-surgical myocardial infarction will be defined by: (1) a rise and/or fall of Troponin T compared to baseline with at least one value above the 99th percentile of the upper reference limit together with evidence of myocardial ischemia with at least one of the following: symptoms of ischemia, ECG changes indicative of new ischemia (new ST-T changes of new LBBB), development of Q waves in the ECG, imaging evidence of new loss of viable myocardium or new regional wall motion abnormality; (2)

sudden unexpected cardiac death involving cardiac arrest often with symptoms suggestive of myocardial ischemia and accompanied by presumably new ST-elevation or new LBBB and/or fresh thrombus on coronary angiography and/or at autopsy, but death occurring before blood samples could be obtained or at time before the appearance of cardiac troponin T in the blood.

Stroke will be defined as a focal, central neurological deficit lasting >72 h which results in irreversible brain damage or body impairment.

Secondary clinical end-points

Clinical outcome at 30 days, including CV death, non-fatal myocardial infarction, revascularization and stroke.

All-cause death

Peri-operative high-sensitive Troponin-T

This will be assessed by measuring serum high-sensitive Troponin-T pre-operatively and at 6, 12, 24, 48, 72 h post coming off cardiac bypass. Following elective CABG ± valve surgery, several studies have demonstrated that peri-operative myocardial injury, indicated by the release of the cardiac enzymes CK-MB, Troponin-T and Troponin-I is associated with worse clinical outcomes following surgery [6–16].

Length of ICU/hospital stay and inotrope score

The length of ICU and hospital stay and the inotrope score are factors which can be influenced by the outcome of surgery and which have an important impact on health-care resources. The inotrope score provides an objective measurement of the requirement of inotropes in the immediate post-operative period. Data on inotrope requirement will be collected daily from the medical drug chart on the ICU. This will be adapted from a study by Ko et al. [44] and will be calculated at 0 (time when coming off bypass), 24, 48 and 72 h after the surgery using the formula below. The inotrope score for the particular time point is calculated as follows: at time 0, the inotrope score will be calculated from the dose of the individual inotropes used at the time of coming off bypass. For 24-, 48- and 72-h time-points, the inotrope score will be calculated from the maximum dose of the individual inotropes used in the previous 24-h period.

Inotrope score =

1. Dopamine + Dobutamine +
2. + [(Adrenaline + Noradrenaline + Isoproterenol + Isoproterenol) × 100]
3. + [Enoximone (or Milrinone) × 15]

All dosages will be in μg/kg/min.

The dosage of Levosimendan will be documented when given.

Remote ischemic preconditioning may impact on these outcome measures by reducing myocardial ischemic injury and preserving left ventricular (LV) systolic function.

Peri-operative AKI

This will be measured by (1) the AKI Score, calculated over the 3-day peri-operative period (Table 1) with serum creatinine measured daily for 3 days (and at 6 weeks and 1-year post-surgery) and urine volumes monitored daily; (2) Neutrophil gelatinase-associated lipocalin (NGAL), a new early marker for AKI, with levels rising rapidly after renal injury [45, 46], measured pre-operatively, 6, 12 and 24 h (post-coming off cardiac bypass).

The six minute walk test (6MWT)

This will be performed at baseline, 6 weeks (in the out-patient clinic follow-up appointment), and at 1-year (in the research outpatient clinic follow-up appointment) post-CABG ± valve surgery. Patients will be instructed to walk as far as possible along a straight, flat hospital corridor in 6 min. The 6MWT will be used to evaluate the effect of RIC on the functional status of patients undergoing CABG ± valve surgery [47]. Shortly after CABG ± valve surgery, functional capacity is significantly reduced, but it rapidly improves after cardiac rehabilitation. This improvement has been found to be independent of age, sex, co-morbidities and baseline functional capacity [47].

Quality of life

The EuroQol EQ-5D Health-Related Quality of Life (HRQOL) questionnaire (http://www.euroqol.org) will be used to assess patients' quality of life post-CABG ± valve surgery [48], at baseline, at 6 weeks (in the surgical out-patient clinic follow-up appointment), at 3 months (by post/e-mail), at 6 months (by post/e-mail), at 9 months

Table 1 The acute kidney injury score

AKI grade	Creatinine criteria	Urine output criteria
1	A rise of >26.4 μmol/L or 150–200% of baseline	<0.5 ml/kg/h for >6 h
2	A rise of 200–300% of baseline	<0.5 ml kg/h for >12 h
3	An increase of >300%; or serum creatinine >354 μmol/L with an acute rise of at least of 44 μmol/L	<0.3 ml/kg/h for >24 h or anuria for 12 h

(by post/e-mail) and at 1-year post surgery (in the research outpatient clinic follow-up appointment). Non-responders will be telephoned.

Left ventricular ejection fraction

A subgroup of 140 patients at two recruitment centres will have a transthoracic echocardiogram performed by bi-planar Simpson's technique and 3D echo techniques, in order to assess left ventricular ejection fraction (LVEF) at baseline and at 1-year post surgery (in the research outpatient clinic follow-up appointment).

Statistical considerations

A detailed statistical analysis plan will be produced prior to unblinding of any data.

Sample size calculation

There will be two arms to the trial: RIC and control. We plan to recruit 1,610 patients through 27 tertiary centres. In the SYNTAX study the MACCE (death, myocardial infarction, revascularization and stroke) rate was 12.4% of patients at 12 months following CABG surgery [49]. However, the patients recruited into the SYNTAX study were generally lower-risk than those to be recruited in ERICCA with a mean EuroSCORE of 3.8, whereas the patients we intend to recruit in our study are higher-risk with EuroSCORE ≥ 5. In another study comprising higher-risk patient defined by them all having left main stem coronary lesions, the MACCE rate (which included some additional neurological criteria) at 1 year was estimated to be 25% [50]. Therefore, for our higher-risk CABG $\pm$ valve surgery patients we have estimated an MACCE rate of 20% at 1 year, which means that to detect a 27% relative reduction in this primary end-point in the RIC-treated group (from 20.0 to 14.6%), with a power of 80% and a significance level of 5%, a sample size of 770 patients will be required for each trial arm (1,540 in total). This was chosen to represent a clinically significant effect, which is less than the reductions in myocardial injury observed in the previously mentioned proof-of-concept clinical studies (i.e. 40–50% reductions in serum cardiac enzymes). To allow for up to 5% dropouts, we plan to recruit 1,610 patients in total (805 patients each arm).

With regards to the ECHO subgroup analysis involving 140 patients in two of the centres, in a previous study [51] IPost was reported to improve LVEF by 7% (absolute increase) from 49 to 56% at 1 year in ST-elevation myocardial infarction patients. In order to detect a smaller mean difference of 5% with a common SD of 10.5%, the sub-study requires 70 patients in each group (140 in total) using 80% power and a 5% significance level.

Statistical analysis

The primary analysis will be a comparison of the 1-year MACCE rate between the RIC and control arms of the trial. Survival analyses techniques will be used for MACCE and other clinical endpoints. Hazard ratios and confidence intervals will be calculated using Cox proportional hazards modeling and Kaplan–Meier curves produced. The assumptions underlying the Cox model will be assessed. In addition, risk differences at 1 year together with 95% confidence intervals will be calculated. Differences in means (continuous variables) together with 95% confidence intervals will be calculated using linear regression models and analysis of covariance techniques where appropriate. Analysis will be by intention to treat on using all available data. We plan to undertake a limited number of subgroup analyses: these will include age, baseline EuroSCORE, LVEF, diabetic status, aortic cross-clamp time, cardiac bypass time and method of cardioplegia (anterograde vs. retrograde blood cardioplegia). All subjects randomized to the study will be analyzed on an intention to treat basis. Data will be validated and the data analysis will take appropriate account of missing values.

Study monitoring

A Trial Steering Committee (TSC) will be responsible for drafting the final report and submission for publication and will meet every 6 months. A Trial Management Group (TMG) will meet weekly during the planning stages of the study and less frequently when the study is actually recruiting. An independent clinical events committee will be convened comprising an independent cardiologist, cardiac surgeon and neurologist. A Data Monitoring and Ethics Committee (DMEC) will meet at the start of the trial to establish a DMEC charter, soon after recruitment has started and then at least annually to determine if there are any unforeseen effects of RIC. This will be the only group, along with the statistician producing the reports for the DMEC, who will see interim analyses by treatment.

Discussion

In the ERICCA trial, we will investigate whether RIC, a non-invasive virtually cost free cardioprotective strategy can improve clinical outcomes at 1 year in higher-risk patients undergoing CABG $\pm$ valve surgery. The risk profile of patients undergoing CABG surgery continues to change with factors such as (a) the aging population (the proportion of patients over 75 years old has increased by more than 4.5-fold over the last decade with the 5-year mortality in this age group being 35%); (b) the increasing

prevalence of diabetes (the proportion of diabetic patients has risen from 15 to 22%, with the operative mortality in this patient group being 2.6%) resulting in an increase in the number of higher-risk patients (defined as an additive EuroSCORE ≥ 5) being operated upon and a corresponding increase in overall operative risk to 5–6% [1, 2]. These higher-risk patients are at a greater risk of peri-operative complications, which are associated with a worse clinical outcome, such as peri-procedural myocardial injury [3], inotropic support post-surgery, significant AKI (up to 34% of patients) [4] and stroke (1–3%) [5]. Peri-operative myocardial injury, as measured by serum CK-MB, Troponin-T or Troponin-I during surgery has been associated with worse clinical outcomes post-surgery [6–16].

The discovery that the RIC stimulus could be reproduced by applying brief episodes of ischemia and reperfusion to the upper or lower limb [52, 53] has facilitated its recent translation from animal studies into the clinical arena. MacAllister et al. [30, 54] were the first to demonstrate the concept of RIC in human volunteers by inflating a blood pressure cuff around the upper arm to 200 mmHg for 5 min and deflating the cuff for 5 min (to induce ischemia and reperfusion, respectively), a cycle which was repeated two more times, with subsequent attenuation of ischemia-induced endothelial dysfunction in the contralateral arm. Cheung et al. [36] applied RIC in children undergoing cardiac surgery using four-5 min cycles of lower limb ischemia and reperfusion, with reduction of myocardial injury, airway resistance and inotrope score. We then demonstrated [34, 35] that three-5 min cycles of upper limb ischemia and reperfusion reduced myocardial injury in adult patients undergoing elective CABG $\pm$ valve surgery.

More recently, RIC using lower limb ischemia/reperfusion has also been reported to induce cardiac, renal and neurological protection in elective surgery for AAA [38, 39] and cervical decompression [40]. Hoole et al. [41] have reported that RIC using brief ischemia and reperfusion of the arm reduced the peri-procedural myocardial injury associated with elective PCI for stable CHD. Botker et al. [42] have recently demonstrated that RIC using four-5 minute cuff inflations/deflations administered in ambulance reduced myocardial infarct size in ST-elevation myocardial infarction patients undergoing primary PCI. In patients undergoing valve surgery alone, RIC comprising three 4-min cycles of cuff inflation and deflation on the lower limb applied at the time of aortic bypass was found to reduce peri-operative myocardial injury although RIC with the same stimulus applied prior to surgery was reported to be ineffective [55]. In patients undergoing off-pump CABG surgery, RIC only resulted in a non-significant 26% reduction in peri-operative myocardial injury as measured by cTnI [56].

Interestingly, not all the studies investigating the effects of RIC in CHD have been positive: Iliodromitis et al. [57] showed more myocardial injury in low-risk patients undergoing elective PCI receiving RIC, although a non-standard RIC protocol was applied, comprising bilateral arm cuff inflation and deflation, and the study may have been underpowered. Moreover, the largest clinical study of RIC in CABG surgery to be published (162 patients randomized to RIC or control) [58] failed to demonstrate any benefits with RIC (three-5-min cycles of inflation and deflation of a blood pressure cuff placed on the upper arm) in terms of peri-operative myocardial injury (cTnT release), ECG changes, cardiac index, inotrope requirements, renal impairment and lung injury. The reason for this negative study is not clear but may be attributable to patient selection (patients with unstable angina were included- these patients may have been inadvertently preconditioned), the RIC stimulus (which was applied in a 'blinded' fashion with the position of the cuff on the arm hidden from view) or concomitant medication (the presence of inhalational anesthetics and intravenous glycerine trinitrate may have interfered with the cardioprotective effect of RIC). In a recently published small study comprising 54 patients undergoing elective CABG under a strict anesthetic regime (the volatile anesthetic isoflurane was given for maintenance of anesthesia until institution of cardio-pulmonary bypass and the intravenous anesthetic propofol was used for induction and following cardio-pulmonary bypass initiation until the end of surgery), Karuppasamy et al. [59] again demonstrated no significant difference in myocardial injury and inflammatory response between RIC and placebo subjects59, although it is possible that the same anesthetics choice and/or their timing might have interfered with RIC [60].

Importantly, in our proposed ERICCA trial, we also intend to include patients undergoing valve surgery in addition to CABG surgery in order to determine whether this higher-risk surgical group may benefit in terms of improved clinical outcomes with RIC. Furthermore, in the previously cited proof-of-concept clinical trials [34, 35] valve surgery patients were included and were demonstrated to benefit from RIC and recent studies specifically investigating ischemic postconditioning have reported benefit in patients undergoing aortic valve surgery [55].

In summary, the ERICCA trial is a large multicentre randomized double blinded clinical trial, which will investigate whether RIC can improve clinical outcomes at 1 year in higher-risk patients undergoing CABG $\pm$ valve surgery. The findings from this trial have the potential to change clinic practice with the introduction of a non-invasive virtually cost-free cardioprotective strategy for improving clinical outcomes in patients with IHD.

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ERICCA Trial

Effect of remote ischaemic preconditioning on clinical outcomes in patients undergoing coronary artery bypass graft surgery

CASE REPORT FORM (CRF)

Patient Initials: ☐☐☐

Date of Birth: ☐☐☐☐☐☐

Hospital Number: ☐☐☐☐☐☐☐☐☐☐

The Efficacy and Mechanism Evaluation programme (www.eme.ac.uk) is funded by the MRC and managed by the NIHR Evaluation, Trials and Studies Coordinating Centre (NETSCC), based at the University of Southampton.

RANDOMISATION FORM (Eligibility)

Patient Initials:				Date of Birth:						

Inclusion criteria:

(Tick)	Yes	No
1. Is the patient undergoing CABG±valve surgery using blood cardioplegia?	☐	☐
2. Is the patient 18 years of age or over?	☐	☐
3. Is the patient's additive EuroSCORE greater than or equal to 5?	☐	☐
Answers to questions 1 - 3 must be "YES" to qualify for the study		

Exclusion criteria:

(Tick)	Yes	No
1. Is there a history of cardiac arrest (on current admission)	☐	☐
2. Is the patient in cardiogenic shock? *	☐	☐
3. Is the patient pregnant?	☐	☐
4. Does the patient have significant peripheral arterial disease affecting the upper limbs?	☐	☐
5. Does the patient have significant hepatic dysfunction (Prothrombin>2.0 ratio)?	☐	☐
6. Does the patient have significant pulmonary disease (FEV1<40% predicted)?	☐	☐
7. Does the patient have renal failure with a GFR<30 mL/min/1.73 m^2?	☐	☐
8. Is the patient on glibenclamide or nicorandil?	☐	☐
9. Is the patient involved in another study which may impact on the ERICCA study?	☐	☐
All answers to questions 1 – 9 must be "NO" to qualify for the study		

*** Definition of cardiogenic shock:**
Systolic blood pressure <90 mmHg for 30 minutes before inotrope/vasopressor administration
Or
Vasopressors or IABP are required to maintain systolic blood pressure >90 mm Hg

RANDOMISATION FORM

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Date informed consent document signed: ☐☐☐☐☐☐

Additive EuroSCORE: ☐☐

ERICCA Study Number: ☐☐☐☐☐

Randomisation date: ☐☐☐☐☐☐

Time of randomisation: ☐☐☐☐

BASELINE DATA

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Date baseline data collected: ☐☐☐☐☐☐

Indications for surgery: **Number of diseased vessels** **1** ☐ **2** ☐ **3** ☐

Valve disease **YES** ☐ **NO** ☐

Sex: **Male** ☐ **Female** ☐

Weight: ☐☐☐ **kg**

Height: ☐☐☐ **cm** ***Body Mass Index will be calculated automatically on the eCRF***

Ethnicity: Caucasian ☐ **Asian** ☐ **Afro-Caribbean** ☐ **Other** ☐ **(Specify below)**

_____

Systolic Blood Pressure: ☐☐☐ **mmHg**

Diastolic Blood Pressure: ☐☐☐ **mmHg**

Heart rate: ☐☐☐ **bpm**

Smoking history: **Current** ☐ **Ex** ☐ **Never** ☐

Shortness of breath in previous month: **YES** ☐ **NO** ☐

If yes, NYHA class (see below): **I** ☐ **II** ☐ **III** ☐ **IV** ☐

Class	Symptoms
I	No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc.
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while **at rest**. Mostly bedbound patients.

BASELINE DATA (Continued)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Angina symptoms in previous month: **YES** ☐ **NO** ☐

If yes, CCS class (see below): **I** ☐ **II** ☐ **III** ☐ **IV** ☐

CCS Functional Classification of Angina		
Class	**Activity Evoking Angina**	**Limits to Physical Activity**
I	Prolonged exertion	None
II	Walking > 2 blocks or > 1 flight of stairs	Slight
III	Walking < 2 blocks or < 1 flight of stairs	Marked
IV	Minimal or at rest	Severe

Left ventricle ejection fraction: **Please enter value or description or not known**

Value available: **YES** ☐ **NO** ☐ **If YES, value** ☐☐ **%**

If NO, description: **Poor** ☐ **Moderate** ☐ **Good/Normal** ☐ **Not known** ☐

Family history of IHD*: **YES** ☐ **NO** ☐

***** Defined as IHD in primary female relative (mother/sister) <65 years old or primary male relative (father/brother) <60 years old

Six minute walk test

Undertaken: Yes ☐ No ☐ If No, give reason:_____

If Yes, total distance walked: ☐☐☐☐ **metres**

Was the test stopped before 6 minutes: Yes ☐ No ☐

If Yes, at what time: ☐☐☐☐ mm:ss

If Yes, give reason:_____

Quality of Life questionnaire (EQ-5D)

Completed: Yes ☐ No ☐

If YES, you will be prompted to enter the EQ-5D data onto the eCRF

BASELINE DATA (Medical history)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Medical history:			
	Yes	**No**	**Year of Diagnosis** *(Enter 0000 if unknown)*
1. Diabetes Mellitus	☐	☐	☐☐☐☐
2. Hypercholesterolaemia	☐	☐	☐☐☐☐
3. Hypertension	☐	☐	☐☐☐☐
4. Previous myocardial infarction	☐	☐	☐☐☐☐
5. Previous PCI	☐	☐	☐☐☐☐
6. Previous CABG	☐	☐	☐☐☐☐
7. Previous stroke	☐	☐	☐☐☐☐
8. Atrial fibrillation	☐	☐	☐☐☐☐
9. Peripheral arterial disease	☐	☐	☐☐☐☐
10. Other **If Yes specify:**_____	☐	☐	☐☐☐☐

BASELINE DATA (Cardiac medication at time of surgery)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Cardiac medication at time of surgery:			
	Yes	**No, stopped prior to surgery**	**No**
1. Aspirin	☐	☐	☐
2. β-blocker	☐	☐	☐
3. Calcium channel blocker	☐	☐	☐
4. Nitrates **If YES specify:**_____	☐	☐	☐
5. Cholesterol-lowering drug **If YES specify:**_____	☐	☐	☐
6. ACE inhibitor/AT2 receptor antagonist	☐	☐	☐
7. Insulin	☐	☐	☐
8. Sulphonylurea	☐	☐	☐
9. Metformin	☐	☐	☐
10. Clopidogrel / prasugrel	☐	☐	☐
11. Warfarin	☐	☐	☐
12. Diuretics	☐	☐	☐
13. Other **If YES specify:**_____	☐	☐	☐

BASELINE DATA (Blood sample collection)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Blood sample collection on the day of surgery (pre surgery):	
For more information please refer to the ERICCA trial blood SOP	**Collected**
BIOMARKERS ☐☐☐☐☐☐ **Date taken**	
Pre RIC/Sham – EDTA tube	☐
Pre RIC/Sham – SST tube	☐
Post RIC/Sham – EDTA tube	☐
Post RIC/Sham – SST tube	☐

STUDY INTERVENTION (On day of cardiac surgery)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Date: ☐☐☐☐☐☐

Intervention: **RIC** ☐ **Sham** ☐ **Not done** ☐

Systolic Blood Pressure: ☐☐☐ **mmHg**

Cuff Site: **Left Arm** ☐ **Right Arm** ☐ **Both** ☐

		(time 24 hr)		
Cuff Inflation	**1:**	☐☐☐☐	**Inflation Pressure:** ☐☐☐	**mmHg**
Cuff Deflation	**1:**	☐☐☐☐		
Cuff Inflation	**2:**	☐☐☐☐	**Inflation Pressure:** ☐☐☐	**mmHg**
Cuff Deflation	**2:**	☐☐☐☐		
Cuff Inflation	**3:**	☐☐☐☐	**Inflation Pressure:** ☐☐☐	**mmHg**
Cuff Deflation	**3:**	☐☐☐☐		
Cuff Inflation	**4:**	☐☐☐☐	**Inflation Pressure:** ☐☐☐	**mmHg**
Cuff Deflation	**4:**	☐☐☐☐		

Has the intervention been completed as intended? YES ☐ *NO* ☐

If "NO" please specify: _____

("NO" should be selected if the duration of any of the cuff inflations is less than 5 minutes)

Expected adverse events: Skin petechiae *YES* ☐ *NO* ☐

Unexpected adverse events: *YES* ☐ *NO* ☐

If YES, please complete relevant SAE or NSAE Form *(See ERICCA Trial protocol for information)*

Form completed by: _____ **Date:** ☐☐☐☐☐☐

Signature: _____

SURGERY DETAILS

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Was CABG performed: Yes ☐ **No** ☐

If No give reason:_____

If Yes: Date of surgery: ☐☐☐☐☐☐ **Time of starting bypass:** ☐☐☐☐ (24 hr)

Consultant: _____

Pre-op ECG: Yes ☐ **No** ☐ **If Yes, date:** ☐☐☐☐☐☐ **Time:** ☐☐☐☐ (24 hr)

Post-op ECG: Yes ☐ **No** ☐ **If Yes, date:** ☐☐☐☐☐☐ **Time:** ☐☐☐☐ (24 hr)

Has the patient had an angiogram in the last 5 days: Yes ☐ **No** ☐

Were any of the following used for anaesthetic induction or maintenance:

	Yes	No		Yes	No		Yes	No
Fentanyl	☐	☐	**Propofol**	☐	☐	**Rocuronium**	☐	☐
Midazolam	☐	☐	**Pancuronium**	☐	☐	**Bevicuronium**	☐	☐
Isoflurane	☐	☐	**Vecuronium**	☐	☐	**Alphentanyl**	☐	☐
Morphine	☐	☐	**Glycopyrrolate**	☐	☐	**Sevoflurane**	☐	☐
Etomidate	☐	☐	**Paracurium**	☐	☐			

Please list any other anaesthetic drugs used:_____

Was levosimendan used: Yes ☐ **No** ☐

Were IV nitrates given during surgery: Yes ☐ **No** ☐

Type of cardioplegia: Anterograde ☐ **Retrograde** ☐

Other ☐ (specify):_____

Bypass time: ☐☐☐☐ hh:mm (elaspsed time)

Cross-clamp time: ☐☐☐☐ hh:mm (elaspsed time)

Time for coming off-bypass: ☐☐☐☐ (24 hr)

Number of grafts: ☐

Valve replaced: YES ☐ **NO** ☐

If YES: Aortic ☐ **Mitral** ☐ **Other** ☐ (specify):_____

Complications: YES ☐ **NO** ☐

If YES specify:

BLOOD SAMPLE COLLECTION (Creatinine)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Blood sample collection (Creatinine):

	Timepoint *	Date	Collected	Sent to local laboratory
Creatinine	Pre-op	☐☐☐☐☐☐	☐	☐
Creatinine	24 hours post-op	☐☐☐☐☐☐	☐	☐
Creatinine	48 hours post-op	☐☐☐☐☐☐	☐	☐
Creatinine	72 hours post-op	☐☐☐☐☐☐	☐	☐

*** Post-op times are recorded from the time of coming off bypass**
Pre-op samples should be taken on the day of surgery

BLOOD SAMPLE COLLECTION (NGAL and Troponin-T)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Blood sample collection (NGAL):

Please note samples should be taken within +/- 1 hour of the specified time

	Timepoint *	Date	Collected
NGAL	Pre-op	☐☐☐☐☐☐	☐
NGAL	6 hours post-op	☐☐☐☐☐☐	☐
NGAL	12 hours post-op	☐☐☐☐☐☐	☐
NGAL	24 hours post-op	☐☐☐☐☐☐	☐

Blood sample collection (Troponin-T):

Please note samples should be taken within +/- 1 hour of the specified time

	Timepoint *	Date	Collected
Troponin-T	Pre-op	☐☐☐☐☐☐	☐
Troponin-T	6 hours post-op	☐☐☐☐☐☐	☐
Troponin-T	12 hours post-op	☐☐☐☐☐☐	☐
Troponin-T	24 hours post-op	☐☐☐☐☐☐	☐
Troponin-T	48 hours post-op	☐☐☐☐☐☐	☐
Troponin-T	72 hours post-op	☐☐☐☐☐☐	☐

*** Post-op times are recorded from the time of coming off bypass**
Pre-op samples should be taken on the day of surgery

BLOOD SAMPLE COLLECTION (Creatinine values)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

These creatinine values should be collected from electronic biochemistry result records

Creatinine values:

Timepoint *	Date	Value (µmol/L)
Pre-op	☐☐☐☐☐☐	☐☐☐☐
24 hours	☐☐☐☐☐☐	☐☐☐☐
48 hours	☐☐☐☐☐☐	☐☐☐☐
72 hours	☐☐☐☐☐☐	☐☐☐☐

*** Post-op times are recorded from the time of coming off bypass**
Pre-op samples should be taken on the day of surgery

POST-OP PERIOD (Urine output)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Urine volumes: These values should be collected daily from patient fluid balance charts

Timepoint * **Date**	**Volume (ml)**	
0 - 24 hours ☐☐☐☐☐☐	**Urine output known?**	YES ☐ ☐☐☐☐ ml NK* ☐ NA* ☐
Was urine output <0.5ml/kg/hr for >6 hrs	Yes ☐	No ☐ NK* ☐
Was urine output <0.5ml/kg/hr for >12 hrs	Yes ☐	No ☐ NK* ☐
Was urine output <0.3ml/kg/hr for all 24 hrs	Yes ☐	No ☐ NK* ☐
24 - 48 hours ☐☐☐☐☐☐	**Urine output known?**	YES ☐ ☐☐☐☐ ml NK* ☐ NA* ☐
Was urine output <0.5ml/kg/hr for >6 hrs	Yes ☐	No ☐ NK* ☐
Was urine output <0.5ml/kg/hr for >12 hrs	Yes ☐	No ☐ NK* ☐
Was urine output <0.3ml/kg/hr for all 24 hrs	Yes ☐	No ☐ NK* ☐
48 - 72 hours ☐☐☐☐☐☐	**Urine output known?**	YES ☐ ☐☐☐☐ ml NK* ☐ NA* ☐
Was urine output <0.5ml/kg/hr for >6 hrs	Yes ☐	No ☐ NK* ☐
Was urine output <0.5ml/kg/hr for >12 hrs	Yes ☐	No ☐ NK* ☐
Was urine output <0.3ml/kg/hr for all 24 hrs	Yes ☐	No ☐ NK* ☐

*** Post-op times are recorded from the time of coming off bypass**

*NK = Not known

*NA – Not applicable

POST-OP PERIOD (Inotrope scores)

ERICCA Study Number: | | | | | |

Patient Initials: | | | **Date of Birth:** | | | | | | |

These INOTROPE values should be collected daily from patient drug chart whilst patient is on ITU. The inotrope score for the particular timepoint is calculated as follows: at time 0, the inotrope score will be calculated from the dose of the individual inotropes used at the time of coming off bypass. For 24, 48 and 72 hour time-points, the inotrope score will be calculated from the maximum dose of the individual inotropes used in the previous 24 hour period. The score will be calculated automatically after entering the individual doses onto the eCRF. **If any of the drugs are not given please enter 0 to the calculation.**

Post-op times are recorded from the time of coming off bypass and 0 hours is defined as the time of coming off bypass.

TIMEPOINT: 0 HOURS

Date | | | | | | |

Dopamine	.	**µg/kg/min**
Dobutamine	.	**µg/kg/min**
Adrenaline	.	**µg/kg/min**
Noradrenaline	.	**µg/kg/min**
Isoproterenol	.	**µg/kg/min**
Enoximone / Milrinone	.	**µg/kg/min**

TIMEPOINT: 24 HOURS

Date | | | | | | |

Dopamine	.	**µg/kg/min**
Dobutamine	.	**µg/kg/min**
Adrenaline	.	**µg/kg/min**
Noradrenaline	.	**µg/kg/min**
Isoproterenol	.	**µg/kg/min**
Enoximone / Milrinone	.	**µg/kg/min**

TIMEPOINT: 48 HOURS

Date | | | | | | |

Dopamine	.	**µg/kg/min**
Dobutamine	.	**µg/kg/min**
Adrenaline	.	**µg/kg/min**
Noradrenaline	.	**µg/kg/min**
Isoproterenol	.	**µg/kg/min**
Enoximone / Milrinone	.	**µg/kg/min**

TIMEPOINT: 72 HOURS

Date | | | | | | |

Dopamine	.	**µg/kg/min**
Dobutamine	.	**µg/kg/min**
Adrenaline	.	**µg/kg/min**
Noradrenaline	.	**µg/kg/min**
Isoproterenol	.	**µg/kg/min**
Enoximone / Milrinone	.	**µg/kg/min**

Intra-aortic balloon pump (as non pharmacological inotropic support): Yes ☐ No ☐

If Yes, for how long: | | | **hours**

CLINICAL DATA UP TO DISCHARGE

ERICCA Study Number: ☐☐☐☐☐

Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Date of discharge: ☐☐☐☐☐☐

If patient dies before discharge please leave this date blank but complete the rest of the form

Death: **Yes** ☐ **No** ☐ *(If "YES" please complete page 26)*

MI: **Yes** ☐ **No** ☐ *(If "YES" please complete page 27)*

Revascularisation: **Yes** ☐ **No** ☐ *(If "YES" please complete page 29)*

Stroke: **Yes** ☐ **No** ☐ *(If "YES" please complete page 30)*

Peri-operative myocardial injury: **Yes** ☐ **No** ☐

Atrial fibrillation: **Yes** ☐ **No** ☐

Acute renal failure: **Yes** ☐ **No** ☐

If Yes, specify: **Haemodialysis** ☐ **Peritoneal dialysis** ☐ **Haemofiltration** ☐

No dialysis or haemofiltration ☐

Significant heart block requiring temporary or permanent cardiac pacing: **Yes** ☐ **No** ☐

Bleeding requiring repeat surgery or drainage: **Yes** ☐ **No** ☐

Length of ITU stay: ☐☐☐ **days** **Length of hospital stay:** ☐☐☐ **days**

Acute Kidney Injury (AKI) Grade: **1** ☐ **2** ☐ **3** ☐ **N/A** ☐
(Please tick N/A if patient does not develop AKI)

AKI Grade	Creatinine criteria	Urine output criteria
1	A rise of >26.4 µmol/L or 150-200% of baseline	<0.5ml/kg/hr for >6 hours
2	A rise of 200-300% of baseline	<0.5ml/kg/hr for >12 hours
3	An increase of >300%; or serum creatinine >354 µmol/L with an acute rise of at least of 44µmol/L	<0.3ml/kg/hr for >24 hours or anuria for 12 hours

Resource diary given to patient: **Yes** ☐ **No** ☐
If no give reason:

ERICCA Study Number: ☐☐☐☐☐

Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Cardiac medication at discharge:		
	Yes	**No**
1. Aspirin	☐	☐
2. β-blocker	☐	☐
3. Calcium channel blocker	☐	☐
4. Nitrates **If YES specify:**_____	☐	☐
5. Cholesterol-lowering drug **If YES specify:**_____	☐	☐
6. ACE inhibitor/AT2 receptor antagonist	☐	☐
7. Insulin	☐	☐
8. Sulphonylurea	☐	☐
9. Metformin	☐	☐
10. Clopidogrel / prasugrel	☐	☐
11. Warfarin	☐	☐
12. Diuretics	☐	☐
13. Other **If YES specify:**_____	☐	☐

FOLLOW-UP - CLINICAL DATA (6 weeks following surgery)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

SINCE DISCHARGE (or date of surgery if still inpatient) **Follow-up date:** ☐☐☐☐☐☐

Death: **Yes** ☐ **No** ☐ *(If "YES" please complete page 26)*

MI: **Yes** ☐ **No** ☐ *(If "YES" please complete page 27)*

Revascularisation: **Yes** ☐ **No** ☐ *(If "YES" please complete page 29)*

Stroke: **Yes** ☐ **No** ☐ *(If "YES" please complete page 30)*

Hospital admission: **Yes** ☐ **No** ☐ **Number of admissions:** ☐☐

(If "YES" please complete the form on page 25 for each admission)

ECG performed: Yes ☐ **No** ☐ **If Yes, date:** ☐☐☐☐☐☐ **Time:** ☐☐☐☐ (24 hr)

Blood sample collection

Creatinine collected: Yes ☐ **No** ☐ **If yes, value:** ☐☐☐☐ **µmol/L**

Six minute walk test

Undertaken: Yes ☐ No ☐ If No, give reason:_____

If Yes, total distance walked: ☐☐☐☐ **metres**

Was the test stopped before 6 minutes: Yes ☐ No ☐ If Yes, at what time? _____

If Yes, give reason:_____

Quality of Life questionnaire (EQ-5D)

Completed: Yes ☐ No ☐ If YES, you will be prompted to enter the EQ-5D data onto the eCRF

Resource diary

Has the resource diary been reviewed: Yes ☐ No ☐

If no give reason:

FOLLOW-UP – Cardiac medication
(6 weeks following surgery)

ERICCA Study Number: ☐☐☐☐☐

Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Cardiac medication at 6 week follow-up:		
	Yes	**No**
1. Aspirin	☐	☐
2. β-blocker	☐	☐
3. Calcium channel blocker	☐	☐
4. Nitrates **If YES specify:**_____	☐	☐
5. Cholesterol-lowering drug **If YES specify:**_____	☐	☐
6. ACE inhibitor/AT2 receptor antagonist	☐	☐
7. Insulin	☐	☐
8. Sulphonylurea	☐	☐
9. Metformin	☐	☐
10. Clopidogrel / prasugrel	☐	☐
11. Warfarin	☐	☐
12. Diuretics	☐	☐
13. Other **If YES specify:**_____	☐	☐

FOLLOW-UP (3 months following surgery)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Quality of Life questionnaire (EQ-5D)

Completed: Yes ☐ No ☐ Date completed or received: ☐☐☐☐☐☐

If YES, you will be prompted to enter the EQ-5D data onto the eCRF

FOLLOW-UP (6 months following surgery)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Quality of Life questionnaire (EQ-5D)

Completed: Yes ☐ No ☐ Date completed or received: ☐☐☐☐☐☐

If YES, you will be prompted to enter the EQ-5D data onto the eCRF

FOLLOW-UP (9 months following surgery)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Quality of Life questionnaire (EQ-5D)

Completed: Yes ☐ No ☐ Date completed or received: ☐☐☐☐☐☐

If YES, you will be prompted to enter the EQ-5D data onto the eCRF

FOLLOW-UP (12 months following surgery)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

SINCE LAST FOLLOW-UP **Follow-up date:** ☐☐☐☐☐☐

Death: **Yes** ☐ **No** ☐ *(If "YES" please complete page 26)*

MI: **Yes** ☐ **No** ☐ *(If "YES" please complete page 27)*

Revascularisation: **Yes** ☐ **No** ☐ *(If "YES" please complete page 29)*

Stroke: **Yes** ☐ **No** ☐ *(If "YES" please complete page 30)*

Hospital admission: **Yes** ☐ **No** ☐ **Number of admissions:** ☐☐☐

(If "YES" please complete the form on page 25 for each admission)

Weight: ☐☐☐ **kg**

Systolic Blood Pressure: ☐☐☐ **mmHg** **Diastolic Blood Pressure:** ☐☐☐ **mmHg**

Heart rate: ☐☐☐ **bpm**

ECG performed: Yes ☐ **No** ☐ **If Yes, date:** ☐☐☐☐☐☐ **Time:** ☐☐☐☐ (24 hr)

Blood sample collection

Creatinine collected: **Yes** ☐ **No** ☐ **If yes, value:** ☐☐☐☐ **µmol/L**

Six minute walk test

Undertaken: Yes ☐ No ☐ If No, give reason:_____

If Yes, total distance walked: ☐☐☐☐ **metres**

Was the test stopped before 6 minutes: Yes ☐ No ☐ If Yes, at what time? _____

If Yes, give reason:_____

Quality of Life questionnaire (EQ-5D)

Completed: Yes ☐ No ☐ If YES, you will be prompted to enter the EQ-5D data onto the eCRF

Resource diary

Has the resource diary been reviewed: Yes ☐ No ☐

If no give reason:

HEART HOSPITAL ECHO SUB STUDY ONLY

Left ventricle ejection fraction: **Please enter value or description or not known**

Value available: **YES** ☐ **NO** ☐ **If YES, value** ☐☐ **%**

If NO, description: Poor ☐ **Moderate** ☐ **Good/Normal** ☐ **Not known** ☐

FOLLOW-UP – Cardiac medication
(12 months following surgery)

ERICCA Study Number: ☐☐☐☐☐

Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

Cardiac medication at 12 month follow-up:		
	Yes	**No**
1. Aspirin	☐	☐
2. β-blocker	☐	☐
3. Calcium channel blocker	☐	☐
4. Nitrates **If YES specify:**_____	☐	☐
5. Cholesterol-lowering drug **If YES specify:**_____	☐	☐
6. ACE inhibitor/AT2 receptor antagonist	☐	☐
7. Insulin	☐	☐
8. Sulphonylurea	☐	☐
9. Metformin	☐	☐
10. Clopidogrel / prasugrel	☐	☐
11. Warfarin	☐	☐
12. Diuretics	☐	☐
13. Other **If YES specify:**_____	☐	☐

FOLLOW-UP – HOSPITAL ADMISSION HISTORY
(Up to 12 months following surgery)

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

This form should be completed for EVERY ADMISSION in the first 12 MONTHS following surgery. Data should be collected from CLINICAL RECORDS
Please complete RELEVANT FORM if indicated
(See ERICCA Trial protocol for information)

Admission date: ☐☐☐☐☐☐ **Discharge date:** ☐☐☐☐☐☐

Primary reason for admission: ☐☐ *(See below for admission codes)*

Death: **Yes** ☐ **No** ☐ *(If "YES", please complete page 26)*

Reason for admission codes:

01: Myocardial infarction

02: Revascularisation

03: Stroke

04: Other CV (specify) _____

05: Other non-CV (specify) _____

CAUSE OF DEATH DATA

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

These data should be collected from CLINICAL RECORDS
Please complete RELEVANT FORM if indicated
(See ERICCA Trial protocol for information)

Date of death: ☐☐☐☐☐☐

Description of cause of death *(from death certificate or relevant medical notes)*:

Was the primary cause of death:

Cardiovascular death ☐

Non cardiovascular death ☐

Unknown * ☐

(* in the absence of an identifiable cause of death)

Autopsy Information *(if available)*:

MI DATA

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

These data should be collected from CLINICAL RECORDS
Please complete RELEVANT FORM if indicated
(See ERICCA Trial protocol for information)

Date of MI: ☐☐☐☐☐☐

Hospital:_____

MI timepoint: **Peri-operative** ☐ **Post-surgical** ☐

NSTEMI or STEMI: **NSTEMI** ☐ **STEMI** ☐

Treatment received:

Other relevant information:

PLEASE PROVIDE SUPPORTING DOCUMENTATION
(clearly marked with ERICCA study number and date of birth)
Please send supporting documents (ECG, blood tests for Troponin T or I or CKMB, copy of hospital admission or GP record) to the Clinical Trials Unit at the LSHTM.

Please see over for definitions

MI DATA (Continued)

Myocardial infarction definition
Myocardial infarction will include both peri-operative myocardial infarction and post-surgical myocardial infarction.

Peri-operative myocardial infarction is defined as follows:
"A rise in Troponin T or I to more than five times the 99th percentile of the normal reference range during the first 72 hours following CABG with or without valve surgery, when associated with the appearance of new pathological Q-waves or new LBBB, or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium".

Post-surgical myocardial infarction is defined as follows:

1. A rise and/or fall of Troponin T or I with at least one value above the 99th percentile of the upper reference limit (URL) together with evidence of myocardial ischaemia with at least one of the following:
    - Symptoms of ischaemia
    - ECG changes indicative of new ischaemia (new ST-T changes of new LBBB)
    - Development of Q waves in the ECG
    - Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

2. Sudden unexpected cardiac death involving cardiac arrest often with symptoms suggestive of myocardial ischaemia and accompanied by presumably new ST elevation or new LBBB and/or fresh thrombus on coronary angiography and/or at autopsy, but death occurring before blood samples could be obtained or at time before the appearance of cardiac troponin T or I in the blood.

STEMI (ST-elevation MI)
New ST elevation at the J-point in two contiguous leads with the cut-off points:
≥0.2 mV in men or ≥0.15 mV in women in leads V_2-V_3 and/or ≥0.1 mV in other leads.

NSTEMI (non-ST-elevation MI)
All MIs that are not STEMI are defined as NSTEMI.

REVASCULARISATION DATA

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

These data should be collected from CLINICAL RECORDS
Please complete RELEVANT FORM if indicated
(See ERICCA Trial protocol for information)

Date of revascularisation: ☐☐☐☐☐☐

Hospital:_____

Procedure: **CABG** ☐ **PCI** ☐

Was the procedure completed as intended: Yes ☐ **No** ☐

Other relevant information:

Revascularisation definition
Repeat revascularisation will be defined as any repeat PCI or CABG with or without valve within the first year post-surgery.

STROKE DATA

ERICCA Study Number: ☐☐☐☐☐

Patient Initials: ☐☐☐ **Date of Birth:** ☐☐☐☐☐☐

These data should be collected from CLINICAL RECORDS
Please complete RELEVANT FORM if indicated
(See ERICCA Trial protocol for information)

Date of stroke: ☐☐☐☐☐☐

Hospital:_____

Clinical features:_____

CT scan confirmed diagnosis: **YES** ☐ **NO** ☐

Haemorrhage or infarct:_____

Other relevant information:

PLEASE PROVIDE SUPPORTING DOCUMENTATION
(clearly marked with ERICCA study number and date of birth)
Please send supporting documents (Copy of medical admission or GP record or result of CT head scan) to the Clinical Trials Unit at the LSHTM.

Stroke definition
Stroke will be defined as a focal, central neurological deficit lasting >72 hours which results in irreversible brain damage or body impairment.

Health Questionnaire

English version for the UK
(validated for Ireland)

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

Self-Care

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

Anxiety/Depression

I am not anxious or depressed ❑

I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today