The effect of remote ischaemic conditioning and glyceryl trinitrate on perioperative myocardial injury in cardiac bypass surgery patients

The ERIC-GTN Trial

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ABSTRACT

BACKGROUND

Due to the aging population and increased prevalence of co-morbidities (such as diabetes, obesity and renal failure), higher risk patients are undergoing coronary artery bypass graft and/or valve (CABG±valve) surgery, increasing the risk of post-surgical complications (such as perioperative myocardial injury/infarction), and worse clinical outcomes. As such, novel cardioprotective strategies are required to protect the heart against perioperative myocardial injury (PMI) during CABG±valve surgery. A number of clinical studies have shown that remote ischaemic preconditioning (RIPC), in which the arm or leg is subjected to cycles of brief ischaemia and reperfusion, by inflating a cuff placed on the upper arm or thigh, can reduce peri-operative myocardial injury (PMI) during CABG±valve surgery. However, not all studies have been positive, and large clinical outcomes studies (ERICCA and RIPHeart) have reported no benefit with RIPC in this clinical setting. The reasons for this are unclear but may relate to co-medications used in this clinical setting including agents such as propofol anaesthesia, and morphine. In this thesis, I investigated whether glyceryl trinitrate (GTN), which is often given as an intra-operative intravenous (IV) infusion, is cardioprotective in its own right, and whether it attenuates the cardioprotective effect of RIPC in patients undergoing CABG±valve surgery.

Primary hypothesis: The effect of RIPC on reducing PMI will be attenuated in the presence of GTN administered as an intra-operative IV infusion in patients undergoing CABG±valve surgery.

Secondary hypothesis: GTN administered as an intra-operative IV infusion will reduce PMI in patients undergoing CABG±valve surgery.

METHODOLOGY

The ERIC-GTN trial (http://www.clinicaltrials.gov: NCT01864252) was a single-site, double-blinded, randomised, placebo-controlled clinical study investigating whether an intra-operative IV GTN infusion attenuates the cardioprotective effect of RIPC in patients undergoing CABG±valve surgery. Consenting adult patients (age >18 years) undergoing elective CABG±valve surgery with blood cardioplegia were eligible for inclusion. Following anaesthetic induction, patients were randomised to receive one of the four treatment groups:

Group 1 - Sham+Saline: a sham RIPC protocol (comprising simulated limb cuff inflations and deflations) followed by an intra-operative intravenous (IV) saline infusion.

Group 2 - Sham+GTN: a sham RIPC protocol followed by an intra-operative IV GTN infusion.

Group 3 - RIPC+Saline: a RIPC protocol (comprising three 5-minute cycles of simultaneous upper arm and thigh cuff inflations/deflations) followed by an intra-operative IV saline infusion.

Group 4 - RIPC+GTN: a RIPC protocol followed by an intra-operative IV GTN infusion.

The primary endpoint was PMI, as quantified by 72 hour area-under-the-curve (AUC) serum high-sensitivity Troponin T.

RESULTS

The intended sample size was 260 patients, but following the results of an interim analysis of 189 patients, the ERIC-GTN trial was stopped. There was no difference in PMI in patients from Groups 1 (Sham+Saline) and 2 (Sham+GTN), suggesting that, in itself, an intra-operative GTN infusion was not cardioprotective. However, patients in Group 3 (RIPC+Saline) did sustain less PMI as evidenced by a 37% reduction in 72

hour AUC Troponin T release, when compared to patients in Group 1 (Sham+Saline), confirming the cardioprotective effects of RIPC. Interestingly, the beneficial effect of RIPC on reducing PMI in Group 3 (RIPC+Saline), was abrogated in the presence of GTN (Group 4, RIPC+GTN), suggesting a negative interaction between RIPC and intra-operative IV GTN infusion.

CONCLUSIONS

The interim analysis of the ERIC-GTN study has shown a negative interaction between RIC and GTN, suggesting that an intra-operative GTN infusion, attenuated the cardioprotective effects of GTN, in terms of reducing PMI in patients undergoing CABG±valve surgery. This finding may, in part, explain the results of the ERICCA trial in which RIPC failed to reduce major adverse cardiovascular events in patients undergoing CABG±valve surgery.

Impact statement:

The findings of the ERIC-GTN trial provides new insights into the interaction between remote ischaemic conditioning (RIC) and intravenous glyceryl trinitrate (GTN) given intraoperatively. GTN is used frequently in open heart surgeries, and establishing the relationship between GTN and RIC is crucial in translating the protective effect of RIC into the clinical setting. The results of this study suggest a negative interaction between RIC and intraoperative GTN, which, in part, could explain the neutral results of the ERICCA trial, in which no significant clinical benefit on patient outcomes was seen as a result of implementing RIC in the clinical setting of open-heart surgery.

The ERIC-GTN trial could help provide information on which patients may benefit from the phenomena known as RIC in the clinical setting i.e. those who are not given intraoperative GTN. Our data adds to emerging studies suggesting other factors can interfere with RIC such as diabetes and certain anaesthetic agents such as propofol.

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Abbreviations:

Abbreviation	Full Term	Page
GTN	Glyceryl trinitrate	1
CABG	Cardiac artery bypass surgery	2
IV	Intravenous	2
PMI	Perioperative myocardial injury.	2
RIPC	Remote ischaemic preconditioning	2
ERICCA	Effect of Remote Ischemic Preconditioning and Outcomes of Cardiac Surgery	2
RIPHeart	Remote ischaemic preconditioning for heart surgery trial	2
AUC	Area under the curve	3
VD	Three-vessel diseases	18
IHD	Ischaemic heart disease	18
PCI	Percutaneous coronary intervention	18
MACCE	Major adverse cardiac and cerebrovascular events	19
ICCF	Intermittent cross-clamp fibrillation	21
MI	Myocardial infarction	23
ECG	Electrocardiogram	24
IMA	Internal mammary artery	24
LAD	Left antero-descending artery	24
LV	Left ventricle	25
LVEF	Left ventricular ejection fraction	25
CPB	Cardio-pulmonary bypass	27

ROS	Reactive oxygen species	27
VF	Ventricular fibrillation	27
IRI	Myocardial ischaemic reperfusion injury	28
STEMI	ST-segment elevation myocardial infarction	28
cTnT	Cardiac troponin	30
ml	Millilitre	31
RISK	Reperfusion injury salvage kinase	32
NO	Nitric oxide	33
mPTP	Mitochondrial permeability transition pore	34
ATP	Adenosine triphosphate	35
IP	Ischaemic preconditioning	36
SAFE	Survivor activator factor enhancement	37
ADP	Adenosine diphosphate	38
L-NAME	L-nitro-arginine methyl ester	40
CGRP	Calcitonin gene-related peptide	41
HIF	Hypoxia-inducible factor	42
SDF	Stromal-derived factor	42
DM	Diabetes mellitus	43
KATP	ATP-sensitive potassium channel	44
RIPerC	Remote ischaemic per-conditioning	45
RIPostC	Remote ischaemic post-conditioning	45
CK-MB	Creatine kinase-muscle/brain	45
HDL	High-density lipoproteins	45

LDH	Lactate dehydrogenase	45
PIS	Patient information sheet	58
NHS	National health services	59
UCLH	University College London Hospitals	60
MAP	Mean arterial pressure	60
IMP	Investigation medicinal product	61
SPC	Summary of product characteristics	61
ICU	Intensive care unit	62
BP	Blood pressure	63
TOE	Transoesophageal echo	64
TTE	Transthoracic echo	64
AKI	Acute kidney injury	65
ITU	Intensive therapy unit	65
AF	Atrial fibrillation	66
CRF	Case report form	68
TMF	Trial main file	68
SD	Standard deviation	70
N	Number	71
BMI	Body mass index	71
HT	Height	71
WT	Weight	71
HTN	Hypertension	73
TIA	Transient ischaemic attack	73
PAD	Peripheral arterial disease	74

β-Blocker	Beta Blocker	75
Ca-Blocker	Calcium channel blocker	75
ACE	Angiotensin-converting enzyme	75
Max	Maximum	76
Min	Minimum	76
OBS	Observation	76
TnT-hs	Troponin T highly sensitive	77
Q-Q Plot	Quantile-quantile plot	80
Conf. Interval	Confidence interval	84
MCAR	Missing completely at random	91
LCx	Left circumflex	99
BNP	Brain natriuretic peptide	102
MV	Mitral valve	102

CHAPTER 1

1.1 Epidemiology of Coronary Artery Disease

Cardiovascular disease is the leading cause of death worldwide. The World Health Organisation has estimated that 2.3 million deaths per year are due to ischaemic heart disease (IHD) (1). Cardiovascular disease accounted for 17.5 million deaths in 2012 (2), of which 7.4 million were due to IHD while 6.7 million were a result of stroke. This corresponds to 13.2% and 11.9%, respectively, of the total number of deaths in the world (3).

Revascularisation, with either percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG) surgery, is the treatment of choice for IHD due to coronary artery disease. The choice of treatment depends mainly on the clinical presentation, symptoms, coronary anatomy, co-morbidities, concomitant medications, prognostic values and patient preference (4).

A review by Sipahi et al. suggested the superiority of CABG over PCI when it comes to long term mortality, myocardial infarction, and repeat revascularisation (5). The SYNergy between percutaneous coronary intervention with TAXus and cardiac surgery (SYNTAX) study (6) aimed to assess the optimum revascularisation treatment for patients with *de novo* 3 vessel disease (VD) or left main (LM) disease (either isolated or in combination with 1, 2, or 3 VD) by randomising patients to either PCI with polymer-based, paclitaxel-eluting TAXUS stents or CABG (6). Meta-analysis by Yusuf et al (4) with a total of 2649 patients showed a reduction in mortality following CABG operation. However; this was not apparent in the first three years of follow-up. After that, risk reduction was significant at 5,7, and 10 years as shown in Figure 1.

Data produced by the 5-year SYNTAX study demonstrated that CABG remains the standard of care for patients with complex coronary disease, driven by favourable rates of MACCE, cardiac death, myocardial infarction, and repeat revascularisation in the CABG group compared with the PCI group. A 5 years follow-up by Mohr et al.(7) showed Kaplan-Meier estimates of MACCE were 26·9% in the CABG group and 37·3% in the PCI group (p<0·0001) as demonstrated in Figure 2. The authors concluded that CABG should remain the standard care for patients with intermediate or high Syntax score, while for patients with less complex disease (low syntax score) or left main coronary disease (low or intermediate SYNTAX scores), PCI would be an alternative option.

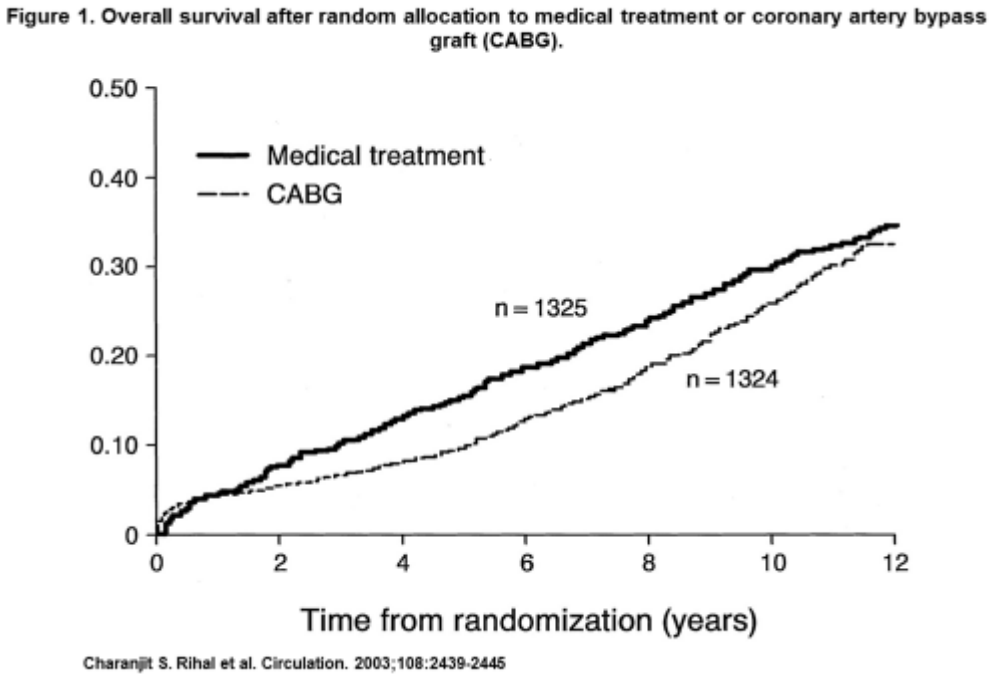


Figure 1. Overall survival after random allocation to medical treatment or coronary artery bypass graft (CABG) surgery (8)

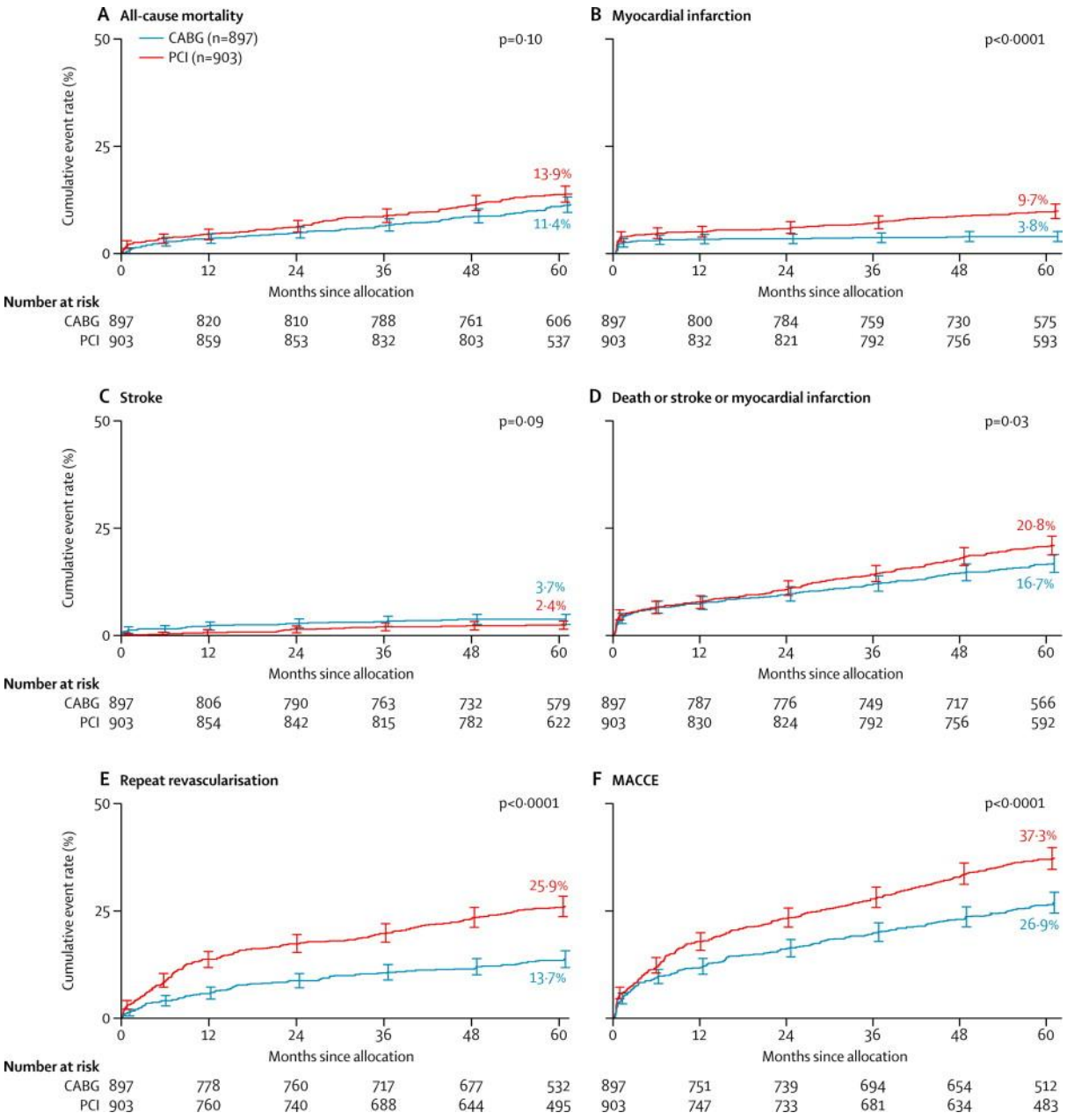


Figure 2. Comparison of CABG and PCI with regards to all-cause mortality, myocardial infarction, stroke, death or stroke or myocardial infarction, repeat revascularisation, MACCE (7) **Kaplan-Meier cumulative event curves at five-year follow-up.**

Any effort to reduce the risk of morbidity and mortality associated with revascularisation is welcomed. In that field, many strategies have been developed including cardioplegia, intermittent cross-clamp fibrillation, bypass machines and improved peri-operative and post-operative care.

1.2 Myocardial preservation techniques

Since the first successful open heart surgery using a cardiopulmonary bypass machine performed by Dr John H. Gibbon, the issue of myocardial protection has been recognised as a crucial element in preserving myocardial function perioperatively, and soon became critical in operation success and patient survival.

Many advances have been achieved in this field. Among them is the technique of aortic cross-clamping, which was introduced to ensure a dry operative field. This also causes global ischaemia, however, albeit transient, which is associated with significant peri-operative myocardial injury (PMI). This led to poorer patient outcomes, so the concept of cardioprotection and PMI emerged as important areas of research.

The first effort to try and protect the heart during CABG surgery was the use of hypothermia (9, 10), but it was soon replaced by "cardioplegic" solutions which were able to induce cardiac arrest due to high concentrations of potassium. However, it was soon evident that myocardial injury using this technique was an issue (11), so it was replaced by hypothermic arrest with continuous coronary perfusion. This was further improved by the induction of ventricular fibrillation, which uses an alternating current to achieve cardiac standstill. It was soon found, however, that this method caused subendocardial ischaemia due to an increase in left ventricular end-diastolic pressure during perfusion; therefore, it was used in combination with intermittent cross-clamping of the aorta, which itself caused transient global ischaemia of the myocardium (12). The resulting technique was termed intermittent cross-clamp fibrillation (ICCF) (13).

A new interest in the cardioplegia technique started to emerge in the 1970s. However, a new form of cardioplegia has since been developed due to the significant myocardial damage caused by the previous method of ICCF, including hypothermia,

normothermia, crystalloid and blood cardioplegia. The development of St. Thomas cardioplegic solution was particularly notable as, while it allows clear visualisation of the surgical field, it also promotes slow recovery of the myocardium and aerobic metabolism, which encourages lactate production and myocardial injury.

Crystalloid solutions contain a high concentration of potassium, a buffer solution such as amino acid and bicarbonate, oncotic agents such as mannitol plus low or high calcium contents.

On the other hand, blood cardioplegia solutions consist of blood and crystalloid solutions at a ratio of 4:1, with high potassium and low calcium concentrations to allow cardioplegia and prevent cardiomyocyte apoptosis and necrosis (14), and glucose to avoid myocardial oedema (15). This gives blood cardioplegia the advantage in cardioprotection due to the ability of these solutions to accelerate the recovery of the myocardium and thus aerobic metabolism, and reduce perfusion damage (15, 16).

However, despite the significant advances in cardioprotection techniques, the PMI sustained during open heart surgery is still substantial, due to the imperfections of the methods mentioned above and the change in the profile of patients requiring these types of operations, who usually have multiple chronic conditions that contribute to the sustained PMI.

1.3 Short and long-term complications in patients undergoing cardiac surgery

Recently, it was noted that the profile of patients undergoing open-heart surgeries is becoming more complicated. Concomitant chronic disease such as diabetes and hypertension, more complex coronary artery disease and associated valve conditions could significantly increase the risk of surgery (17).

Perioperative complications are summarised in the following table:

Table 1.3.1 Perioperative Complications

Myocardial dysfunction	• Acute spasm or occlusion of grafts, prosthetic or paraprosthetic valve regurgitation • Cardiac tamponade, pneumothorax, haemothorax. • Inadequate preload, excessive afterload, impaired ventricular function • Tachy-Brady arrhythmia • Perioperative myocardial infarction (MI)
Vasodilatory shock	• Systemic inflammatory response to ischaemia and reperfusion • Reaction to the cardioplegic solution
Haematological pathologies	• Thrombus or bleeding due to platelet dysfunction, heparin residue or incomplete haemostasis
Pulmonary dysfunction	• Pleural effusion • Atelectasis • Pneumonia • Diaphragmatic incompetence • Intubation issues
Neurological pathologies	• Cerebro-vascular accident • Transient ischaemic attack
Renal Dysfunction.	• Impaired kidney function which could be transient or permanent
Mortality	• Approximately 1% for low risk and 2-5% for the rest of the patients (18, 19)

Multiple factors influence the outcome of open-heart surgery, these factors include:

- **Case Volume**: studies have shown that the higher the volume of cases performed per year by the cardiothoracic centre, the lower the mortality rate, especially among high-risk patients (20, 21).
- **Surgeon experience:** which is independent of the volume of cases a centre would perform but directly correlated with it (22).
- **LV function**: Poor LV function is associated with a 6% increase in the mortality rate (23).

- **Age**: As more elderly patients are being operated on, there is a higher in-hospital mortality risk associated with the procedure (24).
- **Acute Kidney Injury**: Post-operative kidney function deterioration has been shown to be a significant predictor of both mortality and morbidity (25). Also, the mortality rate was shown to be significantly higher in patients requiring renal replacement therapy post-operative compared to patients who did not need such treatment (26).
- **Chronic kidney disease**: which is associated with both short- and long-term post-operative mortality (27, 28).
- **The presence of Q waves on ECG**: where Long-term survival is adversely affected by the appearance of new postoperative Q waves (29).
- **Type of arterial grafting and coronary artery diameter**: The use of arterial grafts such as IMA has been associated with a significant reduction in the rate of hospital stay and long-term mortality (30, 31). Also, the patient with a relatively small diameter of their coronary arteries, especially their LAD, could increase their peri-operative risk (32).
- **Other rare complications**: which could affect the peri-operative mortality in patients undergoing CABG, including gastrointestinal (33), metabolic (34) and haematological (35).

These risk factors have proven crucial in developing formulas capable of predicting mortality risks in patients undergoing cardiac surgery. This enabled both surgeons and patients to weigh the benefits and risks before making an informed decision regarding whether the procedure would be too risky to proceed or not.

The EuroSCORE is an example of these prediction tools. It is widely used in Europe and has recently been adopted worldwide (36). An updated version of EuroSCORE was released in 2011 (37).

Table 1.3.2 EuroSCORE mortality risk prediction algorithm in patients undergoing cardiac surgery

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	1
Extracardiac arteriopathy	Anyone or more of the following: Claudication, carotid occlusion or >50% stenosis, previous or planned intervention on the abdominal aorta, limb arteries or carotids.	2
Neurological dysfunction	Disease affecting ambulation or day to day function	2
Previous cardiac surgery	Requiring opening the pericardium	3
Serum creatinine	>200 mmol/L preoperatively	2
Active endocarditis	The patient is still under antibiotic treatment at the time of surgery	3
Critical preoperative state	Anyone or more of the following: • Preoperative cardiac massage • Preoperative ventilation before arrival in the anaesthetic room • Preoperative inotropic support • Intra-aortic balloon • Preoperative acute renal failure • Ventricular tachycardia or fibrillation	3
Unstable angina	Resting angina requiring I.V nitrate until arrival at the anaesthetic room.	2
LV dysfunction	Moderate or LVEF 30-50% Poor or LVEF <30%	1 3
Recent MI	<90 days	2

Pulmonary hypertension	Systolic pulmonary artery pressure >60mmHg	2
Emergency operation	Carried out on referral before the beginning of next working day	2
Other than isolated CABG	Major cardiac procedure other than or in addition to CABG	2
Surgery on the thoracic aorta	Ascending, arch or descending	3
Post-infarct septal rupture		4

This model could be used as either an additive or logistic risk model: The former could be used to classify patients into high, intermediate or low-risk groups, while the latter is used to calculate the risk of death.

Table 1.3.3 Peri-operative mortality risk undergoing cardiac surgery based on additive EuroSCORE

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

In our study, we have not been recording the EuroSCORE of patients enrolled in the study which could be one of the limitations of this trial.

1.4 Peri-operative myocardial injury

Described as the damage sustained by the myocardium during an invasive procedure or operation, in the environment of cardiac surgery, it is associated with worsening short- and long-term outcomes (38-40).

Several factors can influence the level of troponin released during open heart surgery.

These factors include:

1- Direct damage due to the handling of the heart and other surgical instruments such as the retractor (41).
2- Poor surgical technique, resulting in the aorto-coronary bypass grafting as a result of distal anastomosis or poor harvesting method of the saphenous vein (42) and prosthetic valve incompetence due to poor placement.
3- Systemic inflammatory response to an exogenous substance in the CPB circuit coming in contact with the patient blood. Other factors which could activate the complement cascade include hypothermia, direct surgical trauma and blood loss. Inflammatory mediator release includes cytokines, chemokines, and ROS, all of which lead to a severe inflammatory reaction, the consumption of coagulation factors, microembolisation and multiple-organ failure (43).
4- LV over-distension, especially after CBP, is well-established, leading to retrograde flow to the LV in the presence of AR.
5- Coronary athero-embolisation, either due to an intracoronary thrombus or atherosclerotic debris during coronary manipulation.
6- Ischaemic injury in the setting of both ICCF and cardioplegia as a consequence of intermittent cross-clamping of the aorta carried out during the attachment of the distal end of the graft. The proximal end, however, is constructed after de-clamping and therefore during the reperfusion phase. In ICCF, the ischaemic effect is significant due to a combination of global ischaemia caused by cross-clamping and subendocardial ischaemia due to VF caused by reperfusion. In cardioplegia, more significant global ischaemia is noticed due to the longer cross-clamp time, which is mitigated using cellular protecting solutions. The magnitude of PMI in both ICCF and cardioplegia seems to be equal (44, 45).

7- Myocardial stunning, causing a reversible contractile dysfunction secondary to reperfusion following ischaemia caused by cross-clamping (46).
8- Myocardial ischaemia-reperfusion injury (IRI): The myocardial damage caused by the restoration of blood flow following a period of prolonged ischaemia. In the context of cardiac surgery, it is the result of cross-clamping, intermittent or continuous administration of the cardioplegic solution, cross-clamp fibrillation or a combination of these procedures (47). IRI is recognised as the most relevant potential cause of PMI in cardiac surgery (41).

It is difficult to differentiate between PMI and MI post-op, known as MI type 5, as the mechanism of injury, so the diagnostic tools are similar in both cases.

Recently, the fourth Universal Definition of Myocardial Infarction (48) retains the five types of MI as described in the third Universal Definition of Myocardial Infarction (49), though with modifications.

Type 1 MI: Due to acute coronary atherothrombotic myocardial injury with either plaque rupture or erosion and, often, associated thrombosis. Most ST elevation Mi and many non-ST elevation MI patients fit into this category.

Type 2 MI: Includes patients with evidence of myocardial ischaemia but with no acute coronary atherothrombotic injury. It is usually seen as a mismatch in oxygen demand and supply. It is often regarded as an indication of an underlying coronary artery disease. However, the presence of fixed coronary obstruction is not obligatory, including primary coronary causes such as vasospasm, coronary embolus, and coronary artery dissection. Most of these patients would present as NSTEMI rather than STEMI.

Type 3 MI: Describes the concept that certain people have the characteristics symptoms of myocardial ischaemia but without rise in cardiac troponin levels because the patient succumbs before values are measured or who is stricken by sudden death with evidence of MI by autopsy.

Type 4 and Type 5 MI: Both related to procedural events. Both remained unchanged from the third universal definition of MI, However, it is emphasized that an isolated procedural elevation of cardiac troponin values is indicative of cardiac procedural myocardial injury, that does not alone meet the criteria for percutaneous coronary intervention (PCI)-related type 4a MI, or for coronary artery bypass grafting (CABG)-related type 5 MI.

These categories are applied within 48 hours of the procedure and includes detecting an elevation of cardiac troponin level of more than 10 times the 99 percentile the known upper reference limit for PCI, and greater than 10 times the 99 percentile of the known upper limit for CABG, in addition to new evidence of ischaemia in the form of new ECG changes or cardiac imaging features of myocardial ischaemia, or procedural related complication that may have led to reduced coronary blood flow. This is under the assumption that pre-procedural troponin levels were normal. In cases with elevated pre-procedural troponin levels, the post procedural value most rise more than 20% and also must be 5 or 10 times the upper reference limit.

The clinical significance of raised troponin level post-procedures was not very clear (50), but 5 fold increases in post-procedural troponin where baseline troponin levels were normal, was noted to be prognostically significant.(51, 52)

Similar to PMI, peri-procedural (PCI or CABG) injury could be the result of direct trauma as a result of cardiac manipulation or sewing needles, global or regional ischaemia due to inadequate cardiac protection, microvascular events due to reperfusion, myocardial damage caused by reactive oxygen species (ROS) generation, or failure to reperfuse areas of the heart that are not subtended by grafts (53, 54).

1.4.1 The identification of PMI following cardiac surgery

Similar to MI, type 5 PMI can be diagnosed using a variety of methods in which the most common, least invasive and affordable strategy would be *ECG changes,* which may be suggestive of MI. *Imaging* was developed to assess the diagnosis, and they include:

a) Cardiac magnetic resonance imaging (CMR) which demonstrate a reduction in patient ejection fraction, wall motion abnormalities and evidence of late gadolinium enhancement (55).

b) Radionuclide tracers such as Thallium-201 and Technetium-99m MIBI which allow viable myocytes to be directly imaged (56); in particular, ECG-gated Imaging can provide a reliable assessment of the myocardial function (57).

The issue with these methods is that they often fail to detect a subtle degree of myocardial injury which may have essential prognostic relevance (58) and thus, the emergence of cardiac biomarkers has become crucial in understanding and assessing myocardial damage, and its prognosis (59-61).

1.5 Cardiac Troponin

Cardiac troponin T (cTnT) and troponin I (cTnI) are cardiac regulatory proteins that control the calcium-mediated interaction between actin and myosin (62). Troponin (I)

has not been found outside the myocardium (63), while Troponin T is expressed to a lesser extent in the skeletal muscles; however, the current cTnT assay does not measure skeletal troponin (64). Raised troponin concentration is now widely accepted as a standard biochemical marker for myocardial injury (65).

Following myocardial injury, the first troponin release will be between 3 and 5 hours from membrane destruction, with a second release on the 5th subsequent day due to contractile apparatus damage (66).

Multiple studies have investigated the prognostic values of raised troponin levels in the setting of cardiac surgery. Eigel et al. demonstrated that raised troponin levels post-cardiac surgery were associated with worse clinical outcomes. Intriguingly, their data analysis showed that a cut off at cTnI >0.495ng/L was the best predictor of adverse prognosis (67).

Table 1.3.4 Major studies investigating the prognostic value of raised troponin T post-cardiac surgery

Trial	**Patients recruited and the setting**	**Outcome**
Katherisan.(38) 2004	136 CABG	Troponin T> 1.58ng/ml = 1-year mortality rate predictor
Lehrke (39) 2004	204 CABG	Troponin T>/=0.46ug/L = 4.9-fold increase risk of mortality.
Buse (68) 2009	741 CABG	Troponin T> 0.1µg/L = predictor of 12-month mortality
Nesher (40) 2008	1918 CABG and/or valve surgery	Troponin T>0.8µg/L = increased MACCE

Mohammed (59) 2009	847 CABG	Troponin T levels associated linearly with the length of stay and ventilator hours, and with death, death or heart failure, death or need for vasopressors and the composite of all 3.

1.6 Myocardial ischaemia-reperfusion injury

The concept of reperfusion injury has been a subject of debate for some time now. Some investigators believe that most of the cellular injury happens during the ischaemic phase, while others argued that restoring blood flow could extend myocardial damage through the release of oxygen-derived free radicals, dysregulation of intracellular and mitochondrial calcium, microvascular dysfunction leading to incomplete return of blood flow to areas of the microcirculation (the no-reflow phenomenon), an exaggerated inflammatory reaction involving influx of various populations of immune cells, and delayed cell death due to apoptosis.(69)

Recently, the concept of reperfusion injury has been supported as several pharmacological agents have shown to attenuate the effect of reperfusion injury if applied during the period of reperfusion, such as adenosine and opioids (70, 71). Also, the introduction of novel cardioprotective phenomena termed “post conditioning” which involves reinstitution of blood flow in a stuttering fashion (72), in clear contrast to the ischaemic pre-conditioning phenomena. Finally, the uncovering of the pro-survival signalling pathway termed the reperfusion injury salvage kinase (RISK) (73) in the myocardium has supported the concept of reperfusion injury.

Restoring the blood flow to the myocardium following a prolonged period of ischaemia can start a cascade of events which result in injury (47). In the setting 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 shown that reperfusion injury can account for up to 50% of the final infarct size (47).

The manifestations of reperfusion injury are (74):

1. Reperfusion arrhythmias

2. Myocardial stunning

3. No-reflow phenomenon

4. Lethal reperfusion injury (RI)

1.6.1 Reperfusion arrhythmias

These occur particularly following thrombolysis, PPCI or cardiac surgery, and may manifest as an accelerated idioventricular rhythm (75). The pathophysiology behind reperfusion arrhythmia is linked to the loss of permeability of the mitochondrial membranes, which leads to destabilisation of the action potential across the cell membrane, and could result in arrhythmias in turn (75).

1.6.2 Myocardial stunning

This is a transient, reversible phenomenon caused by persistent anaerobic metabolism following reperfusion and oxidative stress (76).

1.6.3 No-reflow phenomenon or microvascular obstruction

Ischaemic insult can activate the platelet and complement cascade, resulting in severe dysfunction of the resting blood flow in the microvasculature within the ischaemic area (76). 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 (77).

1.6.4 Lethal reperfusion injury (RI)

This refers to cell death caused by the restoration of blood flow to a tissue or organ subjected to a prolonged period of ischaemia (47).

Several mechanisms have been identified as potential causes of RI in animal models; however, translating this into a clinical setting has proven to be challenging (47), thus raising the question of potential disparities between the two models. Therefore, further laboratory testing and clinical trials are needed to explore this fascinating phenomenon and identify clinical practices and agents that are able to reduce RI and thus reduce clinical outcome for patients with IHD.

The mechanism of the pathogenesis of IRI includes:

1) **Oxygen paradox**: Restoration of blood flow to an ischaemic area would activate the xanthine oxidase enzyme, neutrophils, cyclooxygenase, lipoxygenase, and catecholamine oxidation, with subsequent ROS production, cell injury and death (78, 79).

2) **PH paradox**: The normalisation of intracellular PH due to washout of the accumulated lactic acidosis following the restoration of blood flow, in addition to activation of the sodium-hydrogen exchanger and the sodium-bicarbonate exchanger, contributes to lethal RI by inducing the mitochondrial permeability transition pore (mPTP) opening and myocyte hypercontracture (80).

3) **Calcium paradox**: Following of the restoration of blood flow, calcium concentration increases intracellularly as a consequence of the reverse activity of the calcium-sodium exchanger due to sodium-hydrogen exchanger activation, (81), leading to disproportionate myofibril contracture and subsequent myocyte hypercontracture (82).

4) **Inflammation**: The damaged myocardium causes the release of cytokines and complement components, causing neutrophil activation (83).

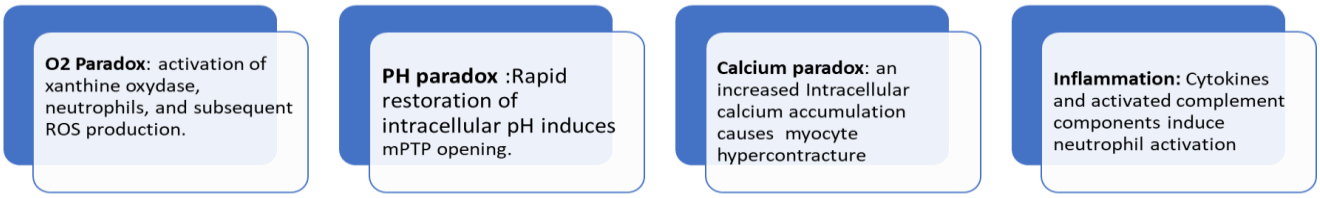


Figure 3. Mechanisms involved in the pathogenesis of lethal RI

It is clear that understanding the mechanism of RI and its contribution to the outcome following MI is crucial to clinical outcome. It also has an impact on the management plan, besides the prompt restoration of blood flow.

1.6.5 Ischaemic Injury

Ischaemic insults caused by the depletion of oxygen causes a significant reduction in oxidative phosphorylation and progressive ATP depletion and thus, activation of the anaerobic glycolytic pathway, which leads to a decrease in intracellular PH and accumulation and a loss of sodium and potassium (84).

This causes activation of the sodium-hydrogen exchanger, which usually favours sodium import and calcium export which regulates intracellular calcium. However, in ischaemic conditions, the sodium-calcium exchanger activity will be reversed, and the calcium concentration will alter, while sodium will be exported extracellularly. At the same time, and in an attempt to ensure ATP production, the cell uses fatty acids from the cellular and mitochondrial membrane, causing a loss of membrane integrity and

further sodium concentration intracellularly. This would worsen the failure of intracellular components and contribute to the transition from reversible to irreversible processes, leading to the initiation of cell necrosis (85). The loss of mitochondrial membrane integrity also induces the intracellular release of enzymes, including cytochromes, caspase and proteolytic enzymes, which are ultimately responsible for cellular apoptosis and autophagy (84, 85).

1.7 Ischaemic Preconditioning (IP)

Several mechanisms have been identified by which the myocardium becomes more resistant to fatal ischaemic injury. These mechanisms include the development of coronary collaterals vessels, myocardium stunning and hibernation, and, crucially, ischaemic preconditioning (IPC) and postconditioning (IPost) (86). Murry et al. first introduced the concept of IPC in 1986 (87), reporting a 75% reduction in infarct size in dogs subjected to four-five minute regional myocardial ischaemia episodes, each followed by a five minute period of reperfusion, before a prolonged period of ischaemia. The cardioprotection induced by IPC has been confirmed in many other studies (88, 89). The same effect has also been noticed in other organs such as the kidneys (90), brain (91), and lungs (92).

Two periods of protection following the insult have been identified: The first is labelled as the “early” or “classic preconditioning period” and is defined as the period immediately following the insult. This period wears off in 1-2 hours (93). A delayed and less protective period is known as the "second window of protection", after 12-24 hours, and can last for up to 72 hours (94).

1.8 Mechanisms of IPC

The production of mediators constitutes the first step in the mechanism of IPC. Potential mediators which have been identified include adenosine (95, 96), opioids (97), acetylcholine (98), catecholamines (99), angiotensin II (100), bradykinin (101), and ROS (102). These multiple mediators bind to specific cell receptors including G-Protein coupled receptor (GPCR) for adenosine (96), bradykinin (101), opioids (103), angiotensin II (100) and other mediators, and growth-factor receptors for insulin, insulin-like growth factor, transforming growth factor, fibroblast growth factor, granulocyte colony stimulating factor, erythropoietin and adipocytokines (73). Other non-receptor-mediated pathways have been described which are involved in IPC triggering, such as heat and stretch (73).

Following receptor activation, a cascade of intracellular transduction pathways activates several pro-survival protein kinases, such as reperfusion injury salvage kinase (RISK) (73), and survivor activator factor enhancement (SAFE) (104), which ultimately lead to inhibition of the opening of the mPTP (105), which leads to the production of anti-necrotic, anti-apoptotic and anti-autophagic effects, thereby reducing cardiomyocyte death (73). The mPTP is a nonspecific high conductance channel situated in the inner mitochondrial membrane, which remains closed during ischaemia and opens in the first few minutes of reperfusion (106).

The opening of the mPTP causes 1) the influx of water and solutes to the mitochondria causing rupture of the outer mitochondrial membrane, the release of intermembrane cytochrome C and the initiation of cell apoptosis and necrosis (107, 108), and 2) the uncoupling of oxidative phosphorylation, leading to ATP hydrolysis, progressive ATP depletion, the collapse of the mitochondrial membrane potential and cardiomyocyte death (107, 108).

The mPTP is known to form F0F1 ATP synthetase (complex V), which in turn synthesises the vast majority of ATP on the inner membrane of the mitochondria, and binds magnesium and ADP/ATP in the presence of low calcium concentrations (109). However, in ischaemic events, the concentration of calcium would increase and cyclophilin-D in the mitochondrial matrix binds to the lateral stalk of complex V, causing formational changes that are responsible for mPTP formation. Interestingly, cyclosporin-A prohibits mPTP opening by preventing cyclophilin-D from binding to F0F1 dimers (110), and also promotes LV recovery, ATP preservation, and MI reduction when given at reperfusion (111). It is understandable that the mPTP would become a central target specifically in understanding the mechanism that is able to prevent the formation of mPTP, thus preventing myocardial cell death (112).

Other significant contributors are the sodium-hydrogen exchanger which reduces swelling and intracellular calcium accumulation (113).

1.9 Remote Ischaemic Preconditioning

Remote ischaemic preconditioning (RIPC) describes the phenomenon by which brief episodes of sub-lethal ischaemia and reperfusion to one organ tissue can reduce IRI in a target organ tissue that is some distance from it (114). Described by Przyklenk et al., this intriguing concept was first introduced through an experiment where anaesthetised dogs with brief occlusions in one myocardial vessel also showed limited infarct size and/or attenuated contractile dysfunction in remote “virgin” myocardium subjected to subsequent sustained coronary occlusion (114). 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. It also

indicated that the protective mechanism could be activated and transported through the heart during the ischaemia/reperfusion period (114).

Subsequent trials showed that RIPC could be elicited when the stimulus is applied to an organ away from the myocardium (115), such as the kidneys (116), intestine (117) and skeletal muscle (118, 119), therefore giving rise to the concept of inter-organ RIPC.

Interestingly, the discovery that RIPC can be elicited through skeletal muscle stimulus led to the broad application of this type of RIPC in humans and clinical settings. Birnbaum et al. (118) were the first group to apply RIPC to an animal model hind limb, where anaesthetised rabbits were randomised into four groups: 1) 30 minute waiting period (controls), 2) 55% to 65% reduction of femoral artery flow (stenosis), 3) electrical stimulation of the gastrocnemius muscle at a rate of one per second, 4) stenosis plus stimulation. This was followed by 30 minutes of coronary artery occlusion and 4 hours of reperfusion. The trial showed that the ratio of infarct size/risk zone was significantly smaller in the stenosis plus stimulation group compared to the control, stenosis, and stimulation groups.

Oxman et al. (119) were the first to apply a non-invasive method of preconditioning stimulus. In their rat model, ischaemia was induced in the hind limbs by using a tourniquet for 10 minutes followed by a period of reperfusion. The trial showed a significant reduction in reperfusion arrhythmias in the preconditioned group. This trial demonstrated the clinical application of non-invasive cardioprotection, which opened the door for a vast number of human trials and applications to make use of this intriguing phenomenon.

1.9.1 Mechanisms of RIPC

Several experiments have been conducted in recent decades to identify the mechanisms underlying RIPC. Most of these trials were exploring the following targets:

- The mechanism triggered by precondition stimulus in the remote tissue.
- The transmission pathway of the protective stimulus from the distal organ tissue to the target organ.
- The cellular mechanism by which the stimulus can confer protection.

1.9.2 Generation of the cardioprotective stimulus in the preconditioned remote organ/tissue

Despite numerous attempts to understand this mechanism through multiple trials, both lab-based and via clinical research, we are still far from understanding the specific mechanism triggered by preconditioning at the target tissue or organ. However, it is generally agreed that a period of IR releases various substances from the ischaemic tissue (120). It has been shown through different studies that multiple mediators, mechanisms, and stimuli are involved at different sites of stimulus (121).

In cardioprotection induced by skeletal muscle, opioids have been shown to play a vital role. In their study, Lu et al. assigned rats randomly to 1 of 7 treatment groups. The morphine and RIPC groups significantly reduced the infarct size compared with the control group. Interestingly, in the group which received RIPC, morphine and naloxone, infarct size was not affected (122).

NO was also shown to be another critical RIPC potential mediator. Chen et al. demonstrated that the protective effect of RIPC in the hind limb of rats was abolished by the NO-synthase antagonist L-nitro-arginine methyl ester (L-NAME) (123). Subsequently, Chen et al. also confirmed the involvement of ROS in skeletal muscle

RIPC by showing that the myocardial protective effects in rats were associated with the increased activity of superoxidase dismutase and glutathione peroxidase, and could be abolished by mercaptopropionyl-glycine, a free radical scavenger (124). Calcitonin gene-related peptide (CGRP) was also found to be involved in early and late conditioning (125).

Adenosine has also been shown to be a potential mediator in cardioprotection by renal ischaemia. This was demonstrated through the abrogation of protective effects in the presence of adenosine receptor antagonists (126), and the increased blood levels of adenosine in preconditioned rabbits (127) and knockout mice (128).

These mediators, once released following IR stimulus from remote tissues or organs, are able to transfer the signal to a distant and target tissue/organ through an as yet not fully understood mechanism. Three main pathways have been identified which could play a part in this process: neural pathway, hormonal pathway, and systemic inflammatory response (120).

In an intriguing experiment, Gho et al. (129) demonstrated that brief ischaemia episodes in remote organs/tissue could protect the myocardium just as effectively as myocardial preconditioning, through transient episodes of anterior mesenteric artery occlusion or left renal artery occlusion, However:

- Transient but not continuous mesenteric occlusion enhanced cardioprotection, suggesting that a period of wash-out is needed for the suggested humoral factor to be produced, and then transmitted to the target tissue/organ.
- The administration of the ganglion blocker hexamethonium abrogated the cardioprotective effects of transient mesenteric ischaemia, giving rise to the possibility of the involvement of a neuronal component.

The **neural pathway** theory suggests that mediators are produced in the distal tissue/ organ, which activate a neural pathway which terminates on the heart (120). On the other hand, the **hormonal theory** stipulates that the ischaemic insult in the remote tissue/organ leads to the formation of mediators locally, which are then released into the circulation, and finally reach the target organ/tissue, possibly during the washout period (120).

The hormonal theory was further supported by Konstantinov (130) and Kristiansen (131), who found that previously preconditioned explanted (and therefore denervated) hearts could be protected against prolonged ischaemia in rats.

However, and in contrast to Gho et al. (129), Wang et al. demonstrated that hexamethonium did not abolish cardioprotection induced by mesenteric ischaemia (132), while Dickson's group "identified" norepinephrine as a potential mediator of RIPC (133). Several studies tried to identify the character of the potential hormonal mediator as a thermolabile, hydrophobic substance with a molecular weight between 3.5 KDa (134) and 15 KDa (135). Other potential humoral factors have been identified as erythropoietin by Diwan et al. (136). Stromal-derived factor-1 (SDF-1α or CXCL12) has been recently linked to cardioprotective activity in mice (137). Hypoxia-inducible factor (HIF) (116) and angiotensin-I (138) have been also investigated as potential humeral factors.

Several other studies have supported Gho's observation that cardioprotection could be blocked by hexamethonium (139, 140). The effort by Ding et al. showed the role of the renal nerve in cardioprotection induced by renal preconditioning (141). Interestingly, the activation of the dorsal motor nucleus of the vagus nerve has been

shown to induce cardioprotection, even in the absence of the remote preconditioning stimulus in the skeletal muscle (142).

In addition, bradykinin, adenosine, and calcitonin gene-related peptide (CGRP) have all been linked to the neural mechanism of RIPC. A higher level of these substances has been reported in preconditioned organs (140, 143). Moreover, they activate the afferent branch of the neural pathway, which is ultimately responsible for the cardioprotective effect. These substances also activate intracellular preconditioning pathways such as A1, BK2, δ1-opioid, k-opioid and angiotensin 1-receptors, which all belong to the GPCR family (120). Crucially, Jensen et al. (144) demonstrated that an interaction between neural (vagal) and hormonal pathways is essential for RIPC. Their trial showed that MI size in isolated naïve rabbit hearts could be reduced by the plasma dialysate obtained by RIPC-treated patients, with or without diabetes mellitus (DM), and without sensory neuropathy, but not by RIPC-treated diabetics with sensory neuropathy.

The systemic anti-inflammatory response is another potential mechanism to explain RIPC signal transmission. This was first proposed by Peralta et al. (145) who demonstrated that hepatic RIPC could produce an anti-inflammatory effect through modulation of the myocardial gene transcription profile with P-selectin up-regulation inhibition, which could ultimately reduce neutrophil migration and oxidative stress and therefore produce the anti-apoptotic effect. This concept was further confirmed via multiple trials including various organs such as the lungs (146) and myocardium (147), and also in human trials (148).

It is feasible that all three mechanisms are responsible for the RIPC effect, as no single mechanism has been fully accepted as being behind it. It is likely that pathways co-exist and interact to deliver the final impact of RIPC (120).

1.9.3 Intracellular signal transduction pathways and end-effectors of RIPC

Studies have demonstrated that PKC activation may mediate the effect of RIPC and that PKC blockers could abolish the same effect (132, 149). In particular, isoform PKC-ε may be responsible for signal transduction followed by bradykinin BK2 (149) and CGRP (150) receptor stimulators. The mito-KATP channel has been shown to have a role in the signalling pathway, as blockers such as the 5-hydroxytryptamine (5HD) blocker abrogated its effect (126). Interestingly, glibenclamide has been shown to block the impact of RIPC and remote ischaemic post-conditioning.(151) Additionally, ROS may also have a significant role in the transduction of the stimulus, as demonstrated by Weinbrenner et al. (152). The RISK pathway involves the pro-survivor protein kinase family, in which the main components are PI3K/Akt and Erk1/2, which may play an essential role in RIPC; this has been demonstrated by the inhibition of p38, Erk1/2 and JNK1/2, which caused the abrogation of cardioprotection following mesenteric preconditioning (153). Also, Erk1/2 stimulation seems to be important in limb preconditioning for both rabbits and humans, as demonstrated by Shimizu et al. (154).

A crucial factor that plays a significant role in RIPC is the end-effector, mPTP. The mPTP has been shown to be the downstream target for mito-KATP activation (155) and the RISK pathway (156). Interestingly, Cao et al. (157) has demonstrated that RIPC cardioprotection was attenuated by the use of the mPTP activator atractyloside and was increased by the use of the mPTP inhibitor cyclosporin A.

1.9.4 Clinical applications of RIPC

Once it was established that animal myocardium could be protected against IRI via transient hind limb IR (118, 119), the clinical translation of this significant discovery was crucial. Two critical features of RIPC were important in transferring it into the clinical setting: 1) **Feasibility**: the finding that a non-invasive technique could result in a reduction of IRI by merely inflating/deflating a blood pressure cough on human volunteers heralded the application of RIPC into several clinical applications (158); and 2) **Flexibility:** The main limitation of IPC is that it has to be applied at a specific time, i.e. before the ischaemic phase and at the onset of the reperfusion phase, and directly to the heart; on the other hand, the remote conditioning stimulus can be applied before (RIPC), after the onset of (RIPerC) or at the end of (RIPostC) ischaemia and to an organ distant from the heart (159).

The first clinical trial investigating the effect of RIPC on the myocardium was conducted by Gunaydin et al. (160) in patients undergoing CABG. The RIPC was applied using a tourniquet around the arm of the patient for 3 minutes followed by a reperfusion period for 2 minutes. This cycle was repeated twice. Markers of PMI were CK-MB and LDH, which were measured before CPB, prior to de-clamping of the aorta and 5 minutes after de-clamping of the aorta. The results showed higher levels of HDL at the second point only, but it is worth mentioning that the trial was underpowered as the team only recruited eight patients. MacAllister's group (158), however, came up with another interesting method to apply RIPC, in which a blood pressure cuff was inflated to 200mmHg on the patient's upper limb. The cuff was inflated for 5 minutes followed by a period of reperfusion. This cycle would be repeated five times before a period of prolonged ischaemia in the contralateral arm through a blood pressure cuff inflated to 200mmHG for 20 minutes. This resulted in an increased response to

acetylcholine in the forearm subjected to prolonged ischaemia as demonstrated by venous plethysmography.

This application of a simple, non-invasive, risk-free technique signalled a new era in research into the potential clinical benefits of RIPC through transient limb IR in the setting of cardiac surgery in the first instance, and more recently, in other settings such as PPCI and non-cardiac surgery. This has been more correctly defined as RIPC as the inflation and deflation of the blood pressure cuff takes place after the onset of ischaemic insult (159), while the stimulus would be called remote ischaemic postconditioning if it was applied at the time of myocardial reperfusion (159).

1.9.5 RIPC and Cardiac Surgery

Following the milestone trial by MacAllister's group (158), Cheung et al. (161) first applied the concept of RIPC with limb IR to the clinical setting, in 17 children who underwent corrective congenital heart disease operations, where RIPC was induced through 4 cycles of 5-minute inflation of a blood pressure cuff applied to the lower limb, followed by 5 minutes of deflation. This showed a reduction in postoperative levels of cTnT, inotropic requirements at 3 and 6 hours, and airway resistance.

The Hatter Cardiovascular Institute at UCL showed for the first time that adult patients undergoing elective CABG surgery and receiving three 5-minute cycles of upper arm IR sustained a significantly lower magnitude of PMI than control patients (162). The relationship between RIPC and cardiac surgery has been investigated extensively, regardless of whether the surgery was in the form of CABG, valve surgery or a combination (Tables 1.9.6.1 and 1.9.6.2). Although most of these trials confirmed the cardioprotective effect of RIPC, several RCTs been neutral, the reasons for which are discussed in Chapter 3.

1.9.6 Drawbacks of the clinical trials of RIPC in cardiac surgery

Matching the animal models with clinical trials has proven to be a challenge, as animal models are subjected to myocardial IRI by direct ligature of the coronary artery, while the majority of studies on RIPC in a clinical setting have been conducted in the context of elective cardiac surgery, where the levels of myocardial damage are expected to be on a magnitude where the clinical benefits from RIPC is usually too small to detect(163). Also, in animal models, no pre-existing conditions are usually taken into consideration, while most CAD patients would have a number of pre-existing conditions such as hypertension, diabetes and hypercholesterolaemia, which may all interact with RIPC (this will be discussed in Chapter 3). Interestingly, it has been demonstrated that patients often suffer pre-infarction angina, which is secondary to transient ischaemia and could, therefore, act as a preconditioning stimulus before an MI. There is now clear evidence that patients who experience angina before their MI do better clinically (164, 165). In this case, a question could be raised as to whether RIPC could add any benefit to an already “preconditioned” myocardium.

Patients recruited to these RCTs are usually on a number of medications, such as anti-diabetic agents, insulin, nitrates and many would receive other agents as part of their in-hospital treatment such as morphine, GTN and clopidogrel. They may receive further agents during surgery, such as propofol, GTN and opioids (comprising morphine and fentanyl). It is worth noting that these agents have been known to mimic IPC (166), and it would be feasible that RIPC would not add further benefit. Furthermore, different surgical techniques in CABG could lead to varying degrees of myocardial damage which are generally represented by cardiac markers (i.e., Troponin I or T) (167).

Other crucial elements of differentiation exist amongst the clinical studies include:

- Type of operation, CABG alone or valve alone or a combination of both, with the subsequent magnitude of PMI sustained.
- The technique of myocardial preservation, whether it is ICCF or cardioplegia.
- The anaesthetic regime used.
- The protective stimulus: The preconditioning stimulus, its timing (before versus after surgical incision), the way it is delivered, and a number of cycles may have a significant effect on the outcome. For instance; in Rahman et al. (168), the RIPC stimulus comprised of three 5-minute cycles of upper-limb 9-cm cuff inflation to 200 mm Hg separated by 5-minute periods of cuff deflation. It is worth mentioning that several trials 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; based on that, we have chosen to use a simultaneous multi-limb preconditioning stimulus to maximise the effect of RIPC in our trial.
- In most cases, the trial's endpoint was PMI measured by troponin or CK-MB release at specific time-points, either as a peak or mean concentration or as a total post-operative AUC. This outcome was associated with short- and long-term morbidity and mortality. However, this represents a surrogate and stronger clinical outcome would be needed to evaluate the effect accurately.

Table 1.9.6.1 Selection of clinical studies investigating the effects of RIPC in CABG

Trial Group	Type of Surgery	Number Recruited	RIPC Stimulus	Myocardial preservation	Myocardial Injury
Hausenloy (162)	Elective CABG	57	Upper-limb ischaemia (3 cycles of 5-min)	Cold-blood cardioplegia and ICCF	Reduced AUC of cTnT (43%)
Rahman (168)	Elective CABG	162	Upper-limb ischaemia (3 cycles of 5-min)	Cold-blood cardioplegia	No difference in cTnT.
Hong (169)	Elective CABG	130	Upper-limb ischaemia (4 cycles of 5-min)	Off-pump	No significant difference in AUC of cTnI
Kottenberg (170)	Elective CABG	72	Upper-limb ischaemia (3 cycles of 5-min)	Cold crystalloid cardioplegia	Reduction of cTnI AUC only when RIPC given with isoflurane and not propofol
Thielmann (171)	Elective CABG	329	Upper-limb ischaemia (3 cycles of 5 min)	Cold crystalloid cardioplegia	Reduce AUC of cTnI. Reduce all-cause mortality

Table 1.9.6.2 Major clinical studies investigating RIPC and valve surgery

Trial Group	Type of surgery	Number recruited	RIC stimulus	Myocardium Preservation	Myocardial Injury
Xie (172)	Elective	73	Upper-limb ischaemia (3x5-min)	Cold blood cardioplegia	44% reduction in TnI AUC
Choi (173)	Complex	76	Lower limb ischaemia (3 x 10-min)	Blood cardioplegia	Significant CK-MB reduction at 24 hours only
Wu (174)	MVR	75	Limb ischaemia pre-conditioning (LIPC-1). LIPC-II (3 x 5 cycles of upper arm ischaemia) plus 2 x 10-min cycles of upper leg ischaemia)		

1.10 Glyceryl Trinitrate

Glyceryl trinitrate is a potent vasodilator, used for over 120 years to treat angina pectoris and, more recently, in the acute and chronic phases of myocardial infarction and in congestive heart failure. GTN molecular formula is C3H5N3O9 or C3H5(NO3)3 as demonstrated in figure 4.

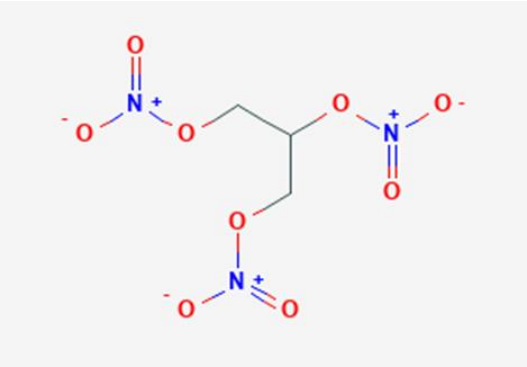


Figure 4. Glyceryl Trinitrate chemical formula (175)

1.10.1 History

GTN was first synthesised by Ascanio Sobrero in 1846. He noticed that GTN can cause headaches which he attributed to vasodilatation. However; it was William Murrell who experimented with the use of nitroglycerin to treat angina pectoris and reduce blood pressure. In 1878 he published his results in the lancet following which the substance was wildly adopted. (176)

1.10.2 Effect and usage

GTN promotes peripheral pooling of blood and decreases venous return, producing a decrease in left ventricular pressure, myocardial wall tension, heart size and, ultimately, myocardial oxygen demand. As a result of the reduction in LVEDP subendocardial perfusion is increased. (177)

Arterial graft spasm can lead to intra and/or post-operative myocardial ischemia and cardiac arrhythmia. GTN infusion has shown to improve the flow in the internal mammary artery. (178, 179)

Treatment of intraoperative hypertension is important to control the myocardial oxygen demand. Nitroglycerin helps control blood pressure by dilating larger arteries without disturbing the metabolic regulation of blood flow in the capillaries. (180)

1.10.3 Pharmacology

Nitroglycerin forms free radical nitric oxide. In smooth muscle, nitric oxide activates guanylate cyclase which increases guanosine 3’5’ monophosphate (cGMP) leading to dephosphorylation of myosin light chains and smooth muscle relaxation. This leads to mainly reduce oxygen demand.

1.10.4 Metabolism

The precise mechanism of GTN metabolism is not clear, however; two pathways have been proposed for its biotransformation. The first is a mechanism-based biotransformation pathway that produces nitric oxide (NO) and contributes directly to vasodilation. The second is a clearance-based biotransformation or detoxification pathway that produces inorganic nitrite anions (NO2-), which does not have apparent cardiovascular effects. Several enzymatic and non-enzymatic systems are capable of metabolising GTN.(181)

1.10.5 Pharmacokinetics in Humans

Regardless of its mode of administration, GTN disappears from the plasma within a few minutes. Its volume of distribution is large, and the systemic plasma clearance usually exceeds the cardiac output. (181)

1.10.6 GTN and Cardioprotection

The prophylactic effect of nitroglycerin infusions during coronary artery bypass surgery was tested by Gallagher et al. (182), in which 41 patients (Group 1) received nitroglycerin, and 40 patients (Group 2) received placebo. All patients received fentanyl for anaesthesia and pancuronium. Mean arterial pressure, pulmonary capillary wedge pressure, heart rate, and cardiac output were measured before and after the induction of anaesthesia, after intubation, before and after chest incision, after sternotomy, after the pericardium was opened and during normothermic cardiopulmonary bypass. However, myocardial ischaemia and infarction were diagnosed from the ECG only. There were no significant differences between groups 1 and 2 in HR, PCWP, or CO seen. Also, there was no difference in the incidence of

ischaemia. In Group 1, nine patients (22%) had ECG changes in ischaemia, while 12 patients in Group 2 (30%) had ischaemia.

Thomson et al. (183) investigated the effect of intravenous nitroglycerin in preventing intraoperative myocardial ischaemia during fentanyl-pancuronium anaesthesia with a randomised, double-blind protocol trial. Continuous ECG recording by a Holter Monitor was used to determine the incidence of ECG changes of myocardial ischaemia during the pre-cardiopulmonary bypass period. Patients in Group 1 (n = 9) received a 0.5 microgram.kg^{-1}.min^{-1} iv NTG infusion 20 min prior to the induction of anaesthesia and throughout the study. Patients in Group 2 (n = 11) received placebo. The incidence of ischaemic ECG changes was virtually identical in Groups 1 (5/9) and 2 (5/11). Interestingly, the authors noted that fentanyl-pancuronium anaesthesia, as administered in this study, was associated with a high incidence of myocardial ischaemia.

Recently, increasing evidence has emerged indicating that NO itself may have an adverse effect on RIPC. Steensrud et al. (184) included 11 groups of rabbits which received placebo (saline), RIPC or adenosine. The groups were pre-treated with the nitric oxide (NO) synthase blocker NG-nitro-L-arginine methyl ester, the NO donor S-nitroso-N-acetylpenicillamine (SNAP), its non-NO-donating derivative N-acetylpenicillamine, or femoral nerve section

Pre-treatment with the NG-nitro-L-arginine methyl ester or N-acetylpenicillamine did not affect the level of protection induced by RIPC or intra-arterial adenosine, but prior femoral nerve transection or pre-treatment with SNAP abolished the cardioprotective effect of intra-arterial adenosine and RIPC.

Crucially, Hauerslev et al. (185) investigated the influence of long-term treatment with glyceryl trinitrate on RIPC. The group studied infarct size (IS) in rat hearts subjected to global ischaemia-reperfusion (I/R) *in vitro*, and endothelial function in healthy volunteers subjected to I/R of the upper arm. In addition to allocated treatment, rats were co-administered with reactive ROS or NO scavengers. Rats and humans were randomised to: 1) control, 2) RIC, 3) GTN, and 4) GTN + RIC. Rats and humans underwent long-term GTN treatment for seven consecutive days, applied subcutaneously or transdermally for 2 hours daily. In rats, RIC and long-term GTN treatment reduced the mean IS (18±12%, p=0.007 and 15±5%, p=0.002) compared to the control (35±13%). RIC and long-term GTN treatment in combination did not reduce IS (29±12%, p=0.55 vs. control), while RIC and long-term GTN prevented the reduction in endothelial function caused by I/R in humans; given in combination, prevention was lost. RIC and long-term GTN treatment both protect against rat myocardial and human endothelial I/R injury through ROS and NO-dependent mechanisms, but when given in combination, RIC and long-term GTN treatment fails to confer protection.

1.11 Conclusions

Remote ischaemic conditioning (RIC) has emerged as a non-invasive, low-cost therapeutic tool using one or more short cycles of limb ischaemia/reperfusion, to reduce perioperative myocardial injury in patients undergoing coronary artery bypass grafting and/or valve surgery.

Most 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. However, several recent trials have failed

to demonstrate this, which may point to certain underlying factors that would compromise the protective effect of RIPC. The role of intravenous glyceryl trinitrate (GTN) therapy administered during cardiac surgery as a cardioprotective agent and whether it interferes with RIC cardioprotection is not clear and forms the bases of investigation in this thesis.

CHAPTER 2

2.1 Aims and Objectives

During cardiac surgery, the myocardium is subjected to PMI, as demonstrated by the rise in cardiac markers such as TnT. Table 2.1.1 shows a number of trials which demonstrated a negative interaction between increased levels of troponin, PMI and post-op complications.

Table 2.1.1 The relationship between raised TnT and PMI.

Trial	Clinical Setting and Patient Number	Outcome
Mohammad et al. (59)	847 patients/CABG only	A linear association was seen between cTnT levels and length of stay and ventilator hours
Adabag et al. (61)	1,186 patients who underwent coronary artery bypass graft surgery (n = 696) or valve surgery (n = 490).	Postoperative cTnI measured 24 hours after heart surgery is independently associated with operative death and perioperative myocardial infarction.
Riedel et al. (60)	Patients with (n = 24) and without (n = 46) P-MI were then followed for three years after CABG surgery to determine the impact of cTn-I–defined P-MI on long-term outcome	P-MI, as defined by cTn-I, is associated with an increased long-term incidence of adverse cardiovascular events.

2.2 Hypothesis

Hypothesis 1: Intra-operative intravenous GTN will reduce PMI in patients undergoing CABG and/or valve surgery.

Hypothesis 2: RIC will reduce PMI in the absence of GTN in patients undergoing CABG and/or valve surgery.

Hypothesis 3: RIC will not reduce PMI in the presence of GTN in patients undergoing CABG and/or valve surgery.

2.3 Overall Aim

The ERIC-GTN trial has been designed to determine whether intra-operatively administered IV GTN is cardioprotective during cardiac surgery and to investigate the interaction between RIC and IV GTN in their cardioprotective effects.

2.4 Objectives

To investigate the effects of remote ischaemic preconditioning and glyceryl trinitrate on peri-operative myocardial injury in patients undergoing cardiopulmonary bypass surgery.

Primary endpoint:

48-hour Area under the Curve (AUC) of high sensitivity Troponin T to assess peri-operative myocardial injury (PMI).

Secondary endpoints:

1. Inotrope/Vasopressor requirements peri-operatively

2. Ventilator dependence postoperatively.

3. The incidence of Acute Kidney Injury assessed using biomarkers (Creatinine).

4. The incidence of Post-operative Atrial Fibrillation.

5. Length of intensive care unit (ICU) stay.

6. Length of Hospital stay.

CHAPTER 3

3.1 Methods and Results

3.1.1 Overview

We conducted a single-centre single-blinded randomised controlled clinical trial between January 2013 and November 2018, to investigate the effect of RIPC and GTN on perioperative myocardial injury.

The RIPC stimulus was induced via multi-limb approach to maximise the effect of RIPC, during anaesthesia induction in patients who were undergoing CABG and/or valve operation who met the inclusion criteria (http://www.clinicaltrials.gov identifier NCT01864252).

3.1.2 Ethical approval and informed consent

The study received ethical approval from the National Health Service Research Ethics Committee, conformed to the spirit and the letter of the Declaration of Helsinki and was conducted in accordance with the principles of Good Clinical Practice under the oversight of University College London Hospital. All participants provided written informed consent.

Eligible patients were approached on the day of their elective admission prior to their surgery, provided with the patient information sheet (PIS) in order for them to have sufficient time to study it, and make an informed decision regarding whether to participate in the trial or not.

3.2 Trial Design

3.2.1 Overall design

The study was designed as a proof-of-concept, single centre trial (started at UCLH NHS Trust then later moved to Bart's NHS Trust). ERIC-GTN was a double-blinded, randomised, placebo-controlled trial. The double-blind nature of the study was achieved using active and placebo treatments. The patient, the anaesthetic and surgical teams, and the trial statistician (data analyst) were blinded to the treatment allocation. A schematic overview of the study design is given in Fig. 3.2.1.

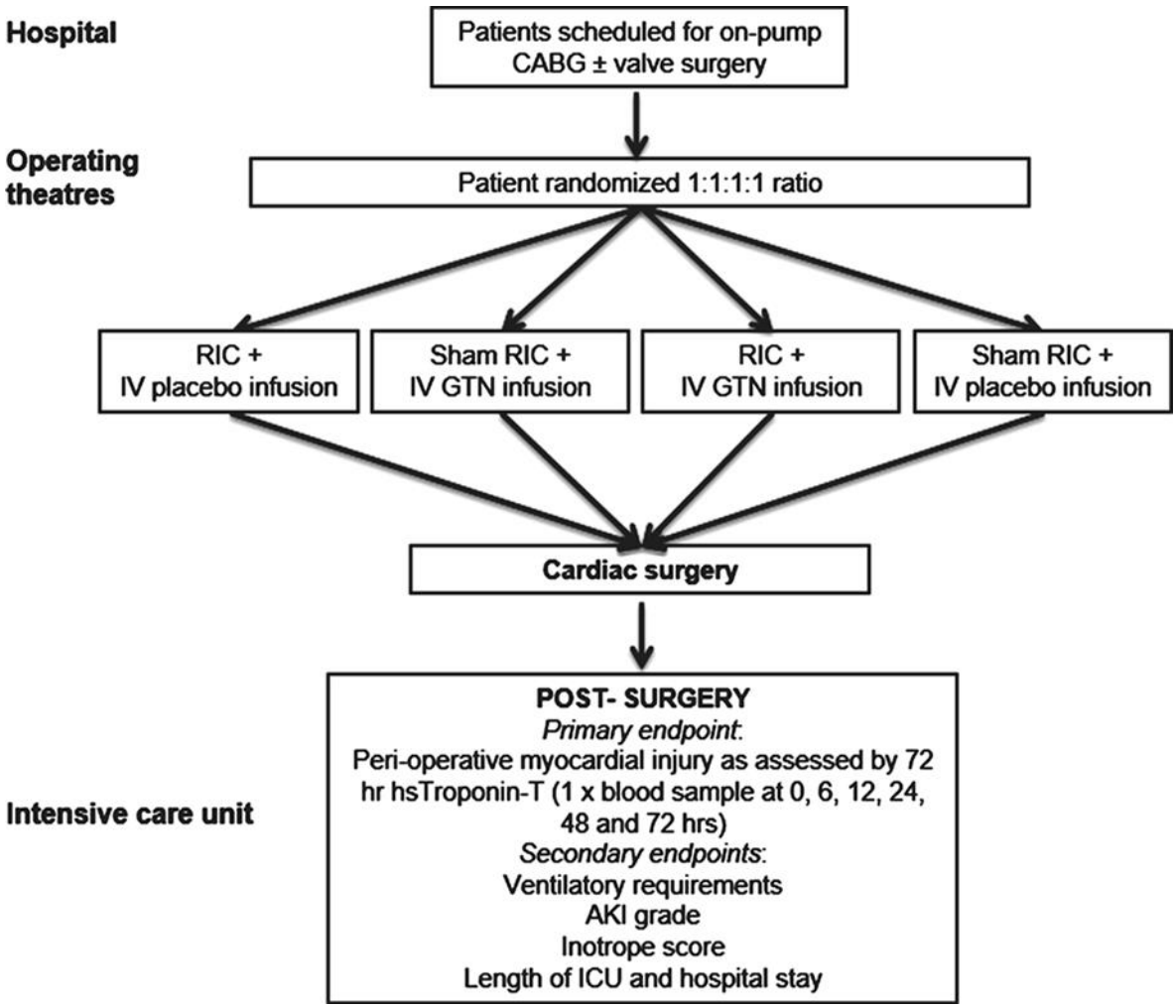


Figure 5. Trial design and flow

The trial included two sub-studies:

- Sub-study 1: An observational study that collected pilot data of cardiac function, at baseline and post-operatively on trans-thoracic echocardiograms, performed on patients who received them as part of the routine follow up.

- Sub-Study 2: Various Cardioprotective markers: Patients were asked to take part in this sub-study which investigated the kinetics of various potential cardioprotective markers, before and after the procedure in patients undergoing CABG.

RIPC or a sham protocol was administered immediately after the induction of anaesthesia and prior to surgical incision. GTN or a saline infusion was started at 2mls/hour once the patient had been transferred from the anaesthetic room to the operating theatre. This was titrated to between 2-5ml/hr before starting bypass, with the aim of maintaining mean arterial pressure (MAP) above 60mmHg at all times.

3.2.2 Patient recruitment

All patients were screened against inclusion and exclusion criteria and recruited if deemed eligible from both sites (UCLH and Bart's), between January 2013 and February 2017.

3.2.3 Inclusion criteria

1. Age > 18 years

2. All patients admitted for on-pump CABG and/or valve surgery.

3. Able to give consent.

3.2.4 Exclusion criteria

1. Allergies to excipients of investigation medicinal product (IMP) and placebo.

2. Chronic renal failure (eGFR<30 ml/min/kg).

3. Severe liver disease.

4. Peripheral arterial disease.

5. Pregnant or lactating women (at the time of IMP administration).

6. Any other contraindications to glyceryl trinitrate as per the summary of product characteristics (SPC)*.

*The SPC states that glyceryl trinitrate should not be used in the following cases:

Known hypersensitivity to nitrates, severe anaemia, severe cerebral haemorrhage, head trauma, uncorrected hypovolaemia and hypotensive shock, arterial hypoxaemia and angina caused by hypertrophic obstructive cardiomyopathy, constrictive pericarditis, pericardial tamponade, toxic pulmonary oedema. Sildenafil potentiates the hypotensive effects of nitrates and its co-administration with glyceryl trinitrate is contraindicated.

3.2.5 Randomisation

The recruited patients were randomised into one of the four arms of the study via the SealedEnvelope™ website. The site was accessed by an authorised study team member at the site, using a unique user id and password.

Upon randomisation, the website assigned a specific code for each patient. This code was checked against the master list provided by the site to see to which arm of the study the patient had been assigned to.

3.2.6 Blinding

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

3.2.7 Intervention: RIPC and sham treatment protocols

RIPC and control protocols were applied after anaesthesia induction and before sternotomy. The RIPC protocol was delivered by simultaneously inflating one blood pressure cuff on one arm to 200 mmHg and another cuff on the leg, and leaving them both inflated for 5 minutes; this was followed by deflating both cuffs to 0 mmHg for 5 minutes. This cycle was repeated three times (i.e., a total of 40 minutes) in total. If the patient's systolic pressure was 185mmHg, the cuff was inflated to 15 mmHg above the systolic blood pressure.

For the control protocol, the two cuffs were simultaneously placed on the upper arm and the upper thigh and left un-inflated for 40 minutes.

Both RIPC and sham protocols were delivered after induction to prevent patient discomfort.

3.2.8 Anaesthetic procedure

The patient received pre-medication with oral temazepam 10-20 mg one hour prior to surgery. The patient was then taken to the anaesthetics room where I.V access was

obtained through the peripheral venous cannula, and an arterial line was also inserted to achieve continuous blood pressure (BP) monitoring.

Anaesthetic induction started with a combination of midazolam, etomidate, propofol, fentanyl and anti-nicotinic agents including rocuronium, vecuronium or pancuronium. Following induction, the trachea was intubated, and mechanical ventilation was started with oxygen with or without air. Trial infusion (GTN, Placebo) would have been started by this stage at 2ml/hr and up-titrated according to MAP (must not be below 60 at any point). 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 electrocardiograms and the nasopharyngeal temperature was continuously recorded.

3.2.9 Surgical procedure

Following anaesthesia, the patient was transferred to the theatre room, and the operation was started with a mid-line sternotomy incision. At that point, left IMA (LIMA) was isolated from the thoracic wall if required, and the great saphenous vein was harvested as necessary.

Standard CPB was employed using a membrane oxygenator and cardiotomy suction, 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, which was achieved with aortic root cross-clamp and either the induction of ventricular fibrillation or the 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 de-clamped and ventricular fibrillation was reverted through a direct current shock.

Cardioplegia was achieved via two different methods:

Antegrade cardioplegia: One part of St. John's Cardioplegia solution mixed with four parts of cold blood was delivered to myocardial cells through the aortic root after aortic cross-clamp, followed by a maintenance cold blood cardioplegia, which was given down the grafts in occluded arteries and into the aortic root every 20-30 minutes. The normal systemic temperature, in this case, would range from 28-32°C.

Antegrade and retrograde cardioplegia: 800 ml of cardioplegia was administered into the aortic root followed by 400ml of retrograde cardioplegia solution given through the coronary sinus, and 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, and the systemic temperature for these patients was 35°C.

3.2.10 Valve replacement/Repair

The decision whether to repair or replace the diseased valve or valves rested with the surgeon, and was usually made before sternotomy by examining various imaging modalities used to assess the valve such as transthoracic echo (TTE), trans oesophageal echo (TOE) and CT scans. A routine TOE was performed by the anaesthetic team prior to sternotomy, to confirm findings and management plan. On several occasions, and based on the findings of the pre-operative TOE, the treatment

for the target valve or valves changed from, for example, repair to replacement and vice versa.

3.3 Secondary Study Endpoints

3.3.1 Acute Kidney Injury (AKI)

AKI in the context of cardiac surgery can occur in 30% of patients. Also, 1-2% of patients can require dialysis which would lead to an 8-fold increase in death (186).

Serum creatinine was measured pre-operatively, and at 6 hours, 12 hours, 24 hours, and 48 hours following the discontinuation of CPB. Hourly urine output and daily urine volumes for the duration of intensive therapy unit (ITU) stay were recorded.

3.3.2 Inotrope requirement

Inotrope requirements are seen as an important indicator of the post-operative recovery period and the surgical outcome, which subsequently have an effect on ICU stay. Inotrope score (187) was recorded in this trial, and was calculated as follows:

Dosages (in µg/kg/min) of [Dopamine + Dobutamine] + [(Adrenaline + Noradrenaline + Isoproterenol + Isoproterenol) x 100] + [(Enoximone + Milrinone) x 15].

3.3.3 Ventilator dependence postoperatively

The duration of endotracheal intubation was noted in hours. Re-intubation rates were calculated by noting down the number of patients requiring re-intubation and comparing this between the four groups.

3.3.4 Incidence of postoperative atrial fibrillation (AF)

New-onset 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) (188).

Postoperative AF is usually secondary to hypovolaemia, electrolyte imbalance, central venous catheters insertion, prolonged aortic cross-clamp times, increased automaticity, increased sympathetic tone and importantly IRI (188). We intended to establish whether RIPC protects patients from new-onset postoperative AF. This was calculated as the incidence of new-onset AF in the first 48 hours after surgery, which was detected by continuous telemetry and ECG.

3.3.5 Length of ITU stay

The length of ITU and hospital stay is a crucial post-operative parameter and represents a significant component of NHS costs and resources. The length of stay in ITU was calculated in days.

3.3.6 Length of stay in hospital

Calculated in days reflecting the total stay for the patients recruited in the hospital.

3.4 Statistical analysis and sample size estimation

3.4.1 Sample size calculation

In a recent trial by our group (Candilio et al. (189)), 178 patients were randomised to receive RIPC or a sham protocol, as described above. A sub-group analysis was performed of patients who had received GTN versus those that did not within the control arm. In total, 65 patients received GTN while 24 did not, interestingly, there

was a significant difference in hsTnT AUC of approximately 20 in this group, which was statistically significant. However, in the groups in which GTN was not given, RIPC resulted in significant protection with small numbers in the groups (n = 21 vs. 35; sham vs. RIPC).

To ascertain the interaction between GTN and RIPC, the ERIC-GTN trial was designed with four groups; the sample size was based on detecting a difference in the means of the RIPC group versus the difference in the means of the GTN group (i.e., the interaction parameter in a linear regression model). We assumed that the AUC is approximately normally distributed, with 80% power, a 5% two-sided significance level and an AUC standard deviation of 21.4 microgram/L (as observed in our pilot data); with 50 patients in each group, we were able to observe a difference of 12 microgram/L or larger in the AUC. Taking into consideration a 5% dropout rate and a 10% crossover rate from the placebo arm to the GTN arm, for safety reasons, we anticipate recruiting 260 patients in total with 65 in each group.

However, and as the main objective of the ERIC-GTN trial has been to determine whether there is an interaction between RIC and GTN, our interim analysis of the ERIC-GTN study showed a negative interaction between the latter and the former, with respect to peri-operative myocardial injury, assessed by Troponin release.

During our final DMEC meeting, the committee concluded that one of the fundamental questions that ERIC-GTN was set out to answer has been fulfilled. This, in addition to other logistical challenges, was enough for the committee to advise stopping the trial; therefore, the recruitment was halted at 192 patients. This issue will be explored further in Chapter 3.

3.4.2 Recruitment Process

As the ERIC-GTN trial was a single site trial, the recruitment process started initially at the Heart Hospital which is part of the UCLH NHS Trust. After the merger between Bart's NHS Trust and UCLH NHS Trust in May 2015, the trial was moved to Bart's Heart Centre, and the recruitment process was resumed there.

The patient screening process was performed by checking the elective cardiac surgery list, which is typically produced at the end of the working day. Screened patients were checked against the inclusion and exclusion criteria. Following that, a member of the trial team approached the patients who met all of the inclusion criteria and none of the exclusion criteria, to offer the PIS of the trial to these patients. The patients were given time to read the document and consider being enrolled in the trial. If the patient decided that they would like to be enrolled, they signed the trial consent form on the following day prior to their surgery. Two copies of the signed consent form were made; the original was filed in the TMF, a copy was given to the patient and the other copy was kept in the patient's medical notes.

The first part of the CRF was completed by a non-blinded member of the trial team during the different stages of the operation (induction, putting the patient on CPB, etc…), while the second part of the CRF was completed by a blinded member of the team. The reason behind having two parts of CRF is that, while the first part could have been completed soon after the end of the surgical procedure, the completion of the second part could have taken several days (until the recruited patient is discharged from hospital). Due to logistic reasons, it would have been difficult to complete the second part by the same non-blinded member of the team, and therefore; another

blinded member completed the second part of CRF. The completed CRF form was then stored in the trial main file (TMF).

3.4.3 Results

In total, 189 patients were successfully recruited between January 2014 and February 2017. One patient with an excessive Troponin T value was excluded, and four patients did not have complete information about the treatment assignment; therefore, data from 184 patients were analysed.

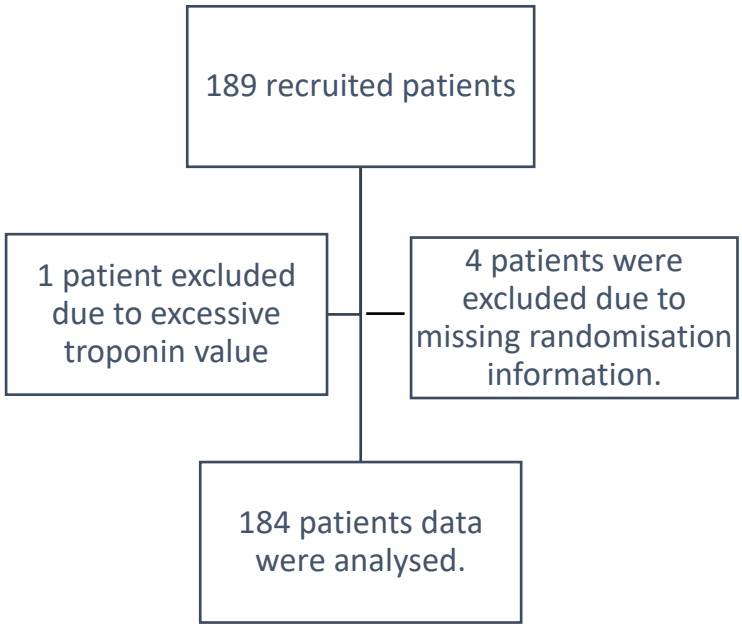


Figure 6. Recruited patients flow

The intervention was completed as per the trial protocol in 174 patients (95.1%) where in 9 patients (4.9%) the intervention was interrupted and was not completed properly. Information for 6 patients were missing.

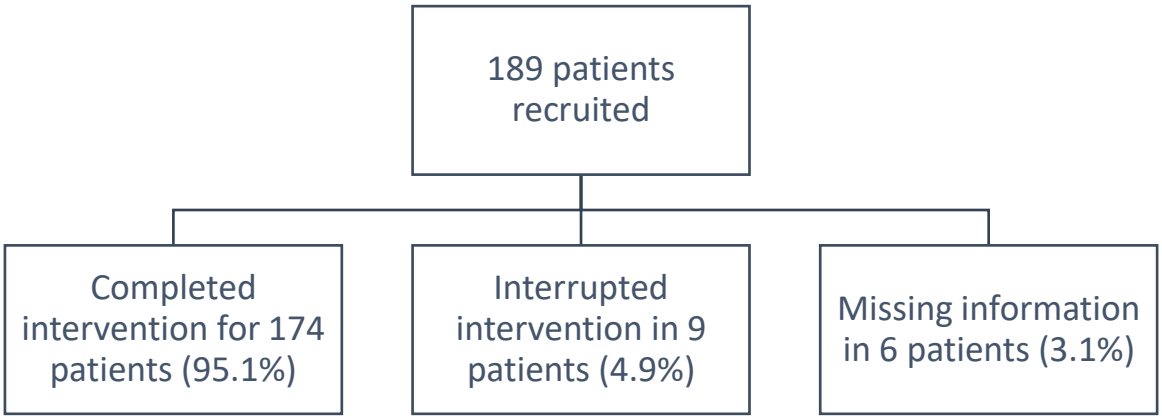


Figure 7. Trial's intervention flow

The investigational medicinal product (IMP) was started at 2ml/hr as stated by the trial protocol. In 181 patients the IMP was started at a minimal dose of 0.1ml/hr, maximum 5.0ml/hr with mean 2.05 and SD 0.5. Data was missing for 8 patients. The maximum dose for IMP for 170 patients was ranging between 1-10 ml/hr, while the calculated mean was 2.98 and the standard deviation was 1.47. The minimum dose for 171 patients was ranging between 0.00-4.1 ml/hr. The calculated mean was 1.89 while the standard deviation was 0.53. In 177 patients, the IMP infusion was completed as per the trial protocol in 157 patients (88.7%), while in 20 patients (11.3%) the protocol was not followed for various reasons including patient's low blood pressure and the anaesthetic team being uncomfortable with the trial's IMP doses.

Number of patients who received propofol as part of the induction regime was 153/177 (86.4%), while the number of patients received propofol as a maintenance infusion was 170/177 (96.0%).

Descriptive statistics were given by the treatment group and alone. Groups were indicated by the variable ***group***, taking values from 1 to 4 corresponding to:

1. **Control** (n=45)
2. **GTN** (n=47)
3. **RIPC** (n=43)
4. **GTN + RIPC** (n=49)

Continuous variables at baseline were found to be well balanced in the four treatment groups.

Table 3.4.3.1 Continuous variables (N, Mean, SD)

Group	Age (years)	WT (kg)	HT (m)	BMI	BP	Pulse/Min	EF%
1 (n=)	44	45	44	44	45	45	33
Mean	68.5	83.3	1.69	29.2	0.6	70.3	58.6
SD	12.1	15.5	0.1	5.6	0.4	10.7	9.1
2 (n=)	46	47	46	46	47	46	34
Mean	66.8	85.3	1.6	29.4	0.6	66.3	58.1
SD	12.3	15.8	0.07	5.1	0.4	11.5	9.5
3 (n=)	39	43	43	43	43	41	30
Mean	65.9	82.2	1.7	28.2	0.7	66.8	57.5
SD	11.5	14.4	0.08	4.7	0.4	10.3	12.6
4 (n=)	49	48	47	47	48	47	32
Mean	67.9	81.6	1.7	28.5	0.6	70.1	54.4
SD	11.7	14.9	0.09	4.6	0.4	12.2	11.3
Total	178	183	180	180	183	179	129
Mean	67.3	83.1	1.7	28.8	0.6	68.4	57.2
SD	11.9	15.1	0.08	5.0	0.4	11.3	10.6

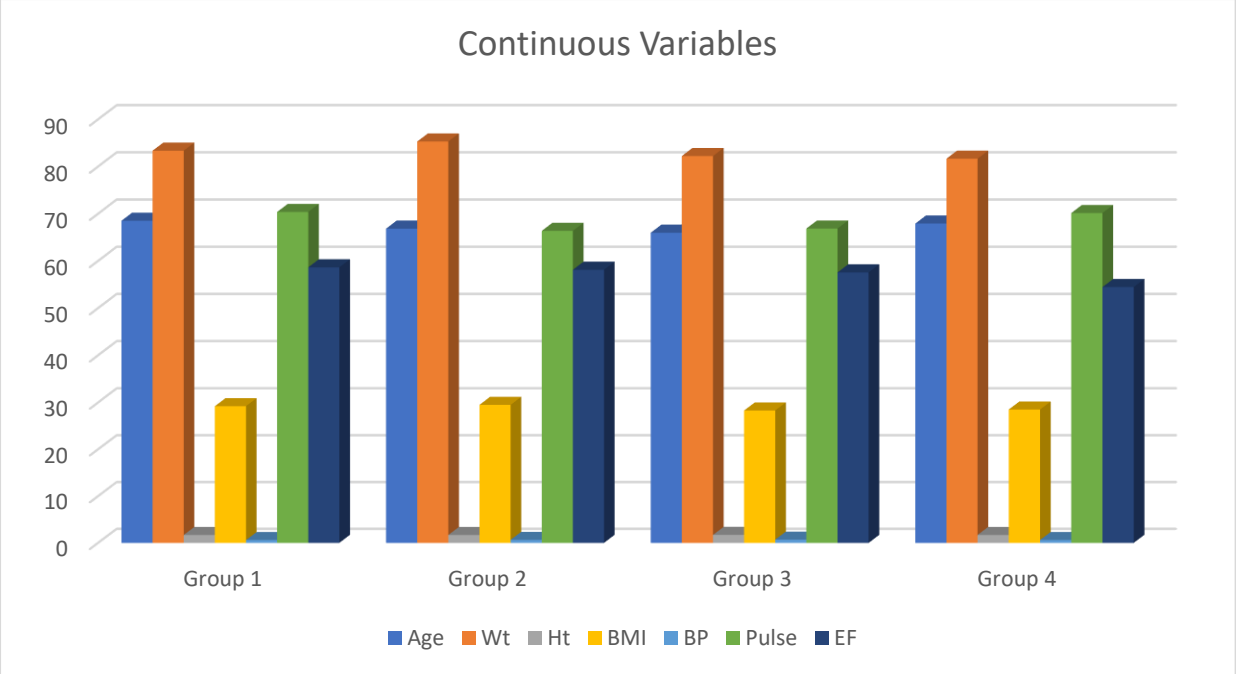


Figure 8. Continuous Variables.

- *Categorical variables*

Categorical variables were well balanced in the four treatment groups. Imbalances in ethnicity depend on the very small number of people in some ethnic groups.

Table 3.4.3.2 Ethnicity

Ethnicity	Group 1	Group 2	Group 3	Group 4	Total
White British	42	37	33	39	151
Ratio %	93.3	80.4	76.7	79.5	82.5
White Irish	0	0	1	0	1
Ratio%	0.00	0.00	2.33	0.00	0.55
White other	1	2	0	5	8
Ratio%	2.2	4.3	0.00	10.2	4.3
White & Black Caribbean	0	0	0	1	1
Ratio%	0.00	0.00	0.00	2.04	0.5
White & Asian	0	1	0	0	1
Ratio%	0.00	2.1	0.00	0.00	0.5
Indian	1	1	2	0	4
Ratio%	2.2	2.1	4.6	0.00	2.2

Bangladeshi	0	1	0	0	1
Ratio%	0.00	2.1	0.00	0.00	0.5
Pakistani	0	1	3	2	6
Ratio%	0.00	2.1	6.9	4.0	3.2
Other Asian	0	2	0	0	2
Ratio%	0.00	4.3	0.00	0.00	1.1
Caribbean	0	1	1	0	2
Ratio%	0.00	2.1	2.3	0.00	1.1
African	1	0	0	0	1
Ratio%	2.2	0.00	0.00	0.00	0.5
Black other	0	0	1	0	1
Ratio%	0.00	0.00	2.3	0.00	0.5
Chinese	0	0	0	1	1
Ratio%	0.00	0.00	0.00	2.0	0.5
Other	0	0	2	1	3
Ratio%	0.00	0.00	4.6	2.0	1.6
Total	45	46	43	49	183
Ratio%	100.00	100.00	100.00	100.00	100.00

- *Binary variables on sex and comorbidities (Percentage, n)*

Table 3.4.3.3 Binary variables on sex and comorbidities (Percentage, n)

Group	Male	DM	Cholesterol	HTN	MI	PCI	CABG	TIA/Stroke
1 (n=)	45	45	45	45	45	45	45	45
	73.3% (33)	24.4% (11)	46.6% (21)	60% (27)	24.4% (11)	13.3% (6)	0	4.4% (2)
2 (n=)	47	47	47	47	47	47	47	47
	85.1% (40)	21.2% (10)	59.5% (28)	63.8% (30)	17.% (8)	17.% (8)	2.1% (1)	4.2% (2)
3 (n=)	43	43	43	43	43	43	43	43
	79% (34)	30.2% (13)	62.7% (27)	69.7% (30)	23.% (10)	20.9% (9)	4.6% (2)	13.9% (6)

4 (n=)	48	48	48	48	48	48	48	48
	77.5% (37)	33.3% (16)	43.7% (21)	60.4% (29)	35.4% (17)	16.6% (8)	4.1% (2)	6. 2% (3)
Total	183	183	183	183	183	183	183	183
	78.8% (144)	27.3% (50)	53.% (97)	63.3% (116)	25.1% (46)	16.9% (31)	2.7% (5)	7.1% (13)

Group	AF	PAD	Smoker
1 (n=)	45	45	40
	17.7% (8)	0	55% (20)
2 (n=)	47	47	43
	6.3% (3)	0	74.4% (32)
3 (n=)	43	43	40
	6.9% (3)	6.9% (3)	67.5% (27)
4 (n=)	48	48	44
	16.6% (8)	2.% (1)	59.% (26)

- *Binary variables on medications at the time of treatment (Percentage, N)*

Table 3.4.3.4 Binary variables on medications at the time of treatment (Percentage, N)

Group	Aspirin	Clopidogrel	B-Blocker	Ca-Blocker	Nitrates	Anti-lipid	ACE	Insulin
1 (n=)	45	45	45	45	45	45	45	45
	57.7% (26)	15.5% (7)	64.4% (29)	31.1% (14)	26.6% (12)	73.3% (33)	64.4% (29)	8.8% (4)
2 (n=)	47	47	47	47	47	47	47	47
	72.3% (34)	25.5% (12)	55.3% (26)	34.0% (16)	31.9% (15)	74.4% (35)	65.9% (31)	02.1% (1)
3 (n=)	43	43	43	43	43	43	43	43
	69.7% (30)	32.5% (14)	69.7% (30)	37.2% (16)	25.5% (11)	86.0% (37)	62.7% (27)	2.3% (1)
4 (n=)	48	48	48	48	48	48	48	48
	54.1% (26)	31.2% (15)	68.7% (33)	27.0% (13)	27.0% (13)	70.8% (34)	58.3% (28)	6.2% (3)
Total	183	183	183	183	183	183	183	183
	63.3% (116)	26.2% (48)	64.4% (118)	32.2% (59)	27.8% (51)	75.9%(139)	62.8% (115)	4.9% (9)

Group	Sulphonylurea	Metformin
1 (n=)	45	45
	13.3% (6)	17.7% (8)
2 (n=)	47	47
	8.5% (4)	14.8% (7)
3 (n=)	43	43
	6.9% (3)	20.9% (9)
4 (n=)	48	48
	6.2% (3)	14.5% (7)
Total	183	183
	8.7% (16)	16.9% (31)

Table 3.4.3.5 Cardioplegia Technique

Cold	Warm	Antegrade	Retrograde	Crystalloid	Blood	Missing
116/173 (67.1%)	43/173 (19.7%)	145/173 (83.8%)	27/173 (15.6%)	6/173 (3.5%)	142/173 (82.2%)	16 (8.5%)

Table 3.4.3.6 Intermittent Cross-Clamp Fibrillation

Total Number	Yes	No	Missing
172	26/172 (15.1%)	146/172 (84.9%)	17 (9.0%)

- Troponin T values

TroponinT values were measured at each time point.

Table 3.4.3.7 Troponin values at each time point.

VARIABLE	OBS	MEAN	SD	MIN	MAX
TNT-HS (0H)	164	59.9	177.8	3	1201
TNT-HS (6H)	147	895.1	873.7	169	6474
TNT-HS (12H)	158	733.1	714.8	12	5252
TNT-HS (24H)	153	529.8	527.1	123	4323
TNT-HS (48H)	127	408.7	354.9	92	2607

Missing data was an issue. Data were noticeably missing at the 48-hour time-point for multiple reasons, including the fact that most of the recruited patients would have been moved out of the intensive care unit to the medical ward. Therefore, patients would not be monitored on a one-to-one basis, and taking research blood samples could not be accommodated by the busy medical staff on the wards on some occasions. Another important factor is the lack of trial staff able to take blood samples at the correct point in time, in case medical staff were not able to. The trial team would have been more involved in recruiting another patient for the trial at that stage.

A closer look at Troponin T summary statistics (**N, mean, SD**) within each group is shown in the table below.

Table 3.4.3.8 Troponin T statistical summary according to treatment group

Group	TnT-hs (0H)	TnT-hs (6H)	TnT-hs (12H)	TnT-hs (24H)	TnT-hs (48H)
1 (n=)	41	37	40	36	31
Mean	17.4	1003.4	806.9	672.6	455.2
SD	24.8	820.0	688.0	759.2	472.9
2 (n=)	39	36	37	37	28
Mean	19.6	877.7	739.5	448.0	418.9
SD	19.6	937.5	821.5	396.6	318.8
3 (n=)	41	33	40	40	31
Mean	97.5	647.5	569.7	412.7	308.4
SD	221.1	395.9	380.4	266.0	214.9
4 (n=)	43	41	41	40	37
Mean	101	1011.8	814.5	594.0	446.0
SD	262.4	1100.3	869.8	553.3	357.2
Total	164	147	158	153	127
Mean	59.9	895.1	733.1	529.8	408.7
SD	177.8	873.7	714.8	527.1	354.9

The **complete data for Troponin T values** (i.e. those available to calculate the Area under the Curve) is shown in the table below.

Table 3.4.3.9 Complete data available to calculate the area under the curve

GROUP	TNT-HS (0H)	TNT-HS(6H)	TNT-HS (12H)	TNT-HS (24H)	TNT-HS (48H)
1	20	20	20	20	20
MEAN	14.7	1036.3	873.9	759.7	497.7
SD	13.4	954.6	865.1	973.5	570.7
2	18	18	18	18	18
MEAN	23.7	1004.9	887.1	518.4	389.0
SD	26.0	1214.5	1065.3	514.9	296.4
3	26	26	26	26	26
MEAN	43.8	620.0	502.6	351.4	270.3
SD	86.9	392.9	370.8	236.0	165.8
4	27	27	27	27	27
MEAN	135.5	1115.4	891.1	605.4	493.0
SD	316.7	1293.5	1020.3	576.2	389.6
TOTAL	91	91	91	91	91
MEAN	60.6	934.6	775.5	549.5	409.8
SD	183.7	1019.3	861.1	619.5	382.6

Using complete data, it was possible to get the graphical representation of the MEAN areas under the curves under each treatment group. SD was considered relevant, meaning that individual curves could vary consistently.

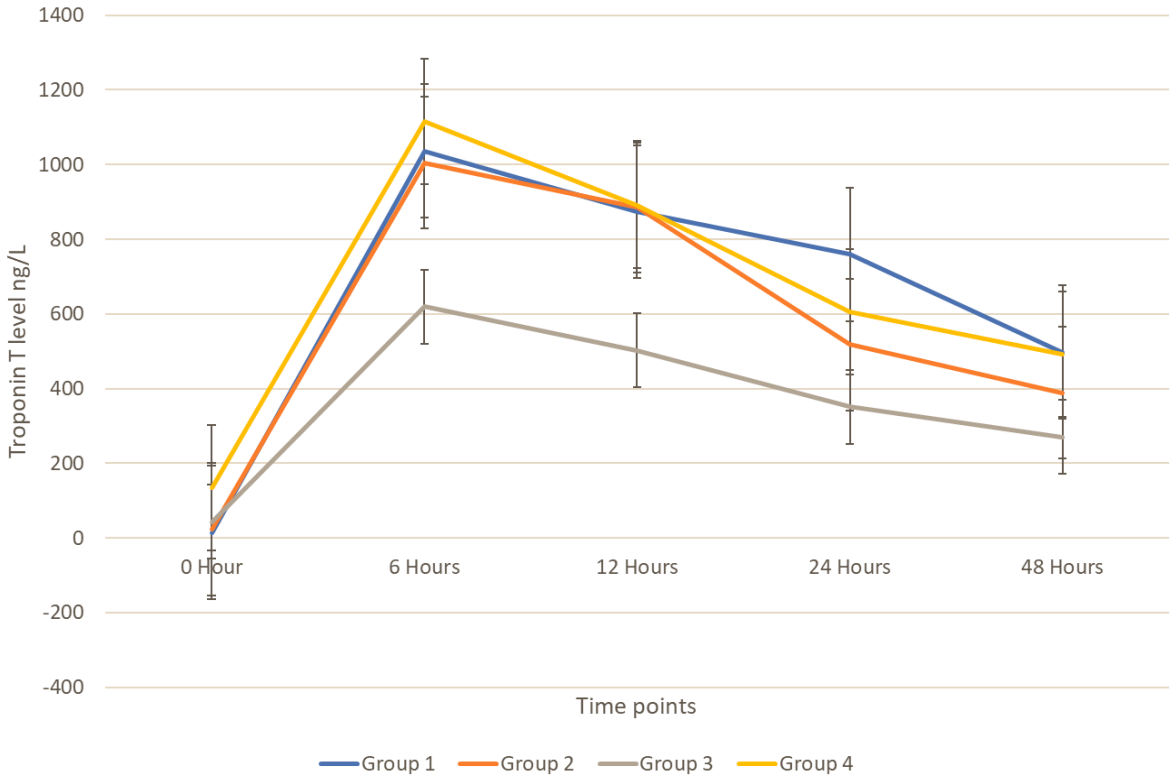


Figure 9. The graphical representation of the MEAN areas under the curve under each treatment group (using the complete data).

The following table shows that the mean AUC was visually smaller for group 3 (RIPC only). The significance of this will become clear towards the end of this chapter.

Table 3.4.3.10 AUC for all groups

Group	Number	Mean	SD
1	20	33775.0	37203.5
2	18	28085.3	29317.0
3	26	17945.7	11359.2
4	27	31933.6	31819.0
Total	91	27580.6	28704.6

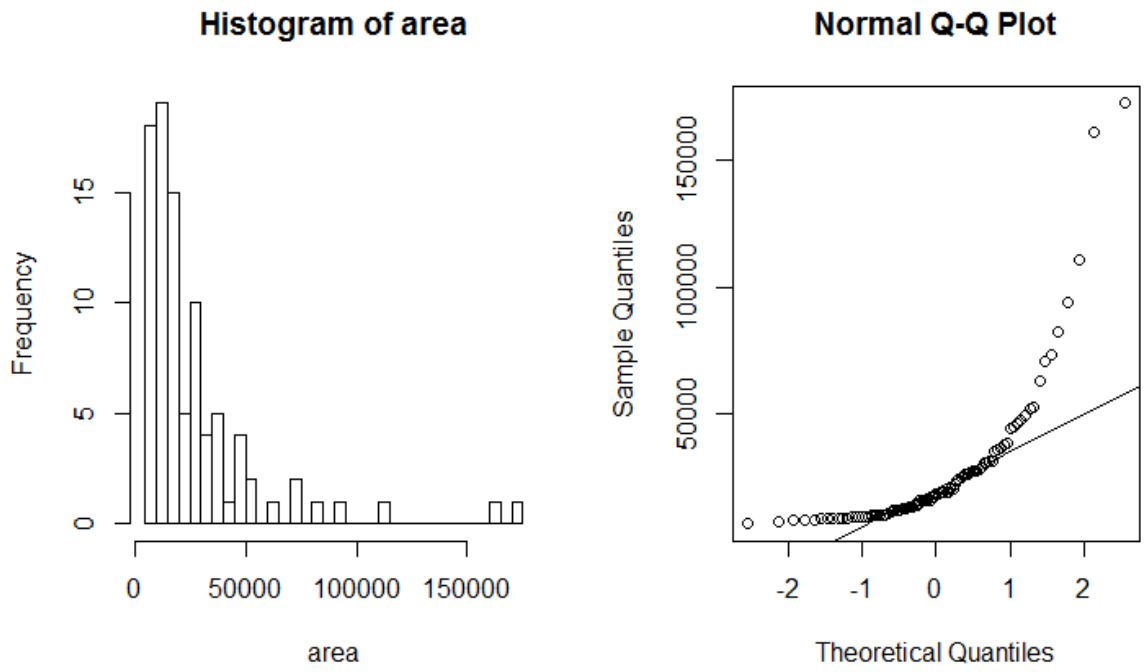


Figure 10. AUC using histogram of area and normal Q-Q plot.

Examining the graph above, the AUC did not appear to be normally distributed; however, the **log (AUC)** seemed to better comply with the following assumption:

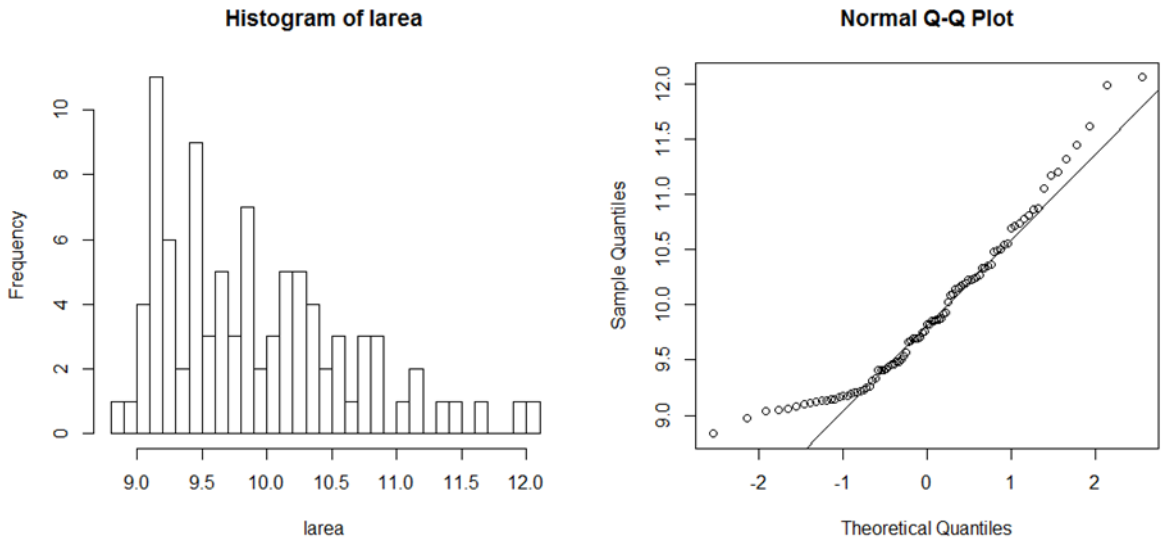


Figure 11. Log transferred AUC

3.4.4 Regression Analyses

- ANOVA results gave significance for the main effects of the treatments and the interaction between them, indicating that the treatment was effectively *averaging* the possible values (in this case 2) of the other treatment.
- Regression tables coefficients were summed up and then exponentiated to obtain geometric expected values for AUC in the four treatment groups.

This distinction was meaningful to interpret the tables with pairwise comparisons. Those tables contained the main effects on the top and group comparisons on the bottom rows. If the interaction term in ANOVA/regression tables (test is the same) was significant, the main effects would have lost their meaning since, although an average effect could have been calculated, it would have been misleading: the effect of one treatment changes (see the graphs with the crossing lines below) according to the administrative status of the other treatment. In these cases, it was better to look at group comparisons instead.

Anova is usually used to compare the means for combinations of two independent binary variables. In this case interaction was significant, while the main effect was not. Histogram and QQ plots of residuals showed an acceptable departure from normality.

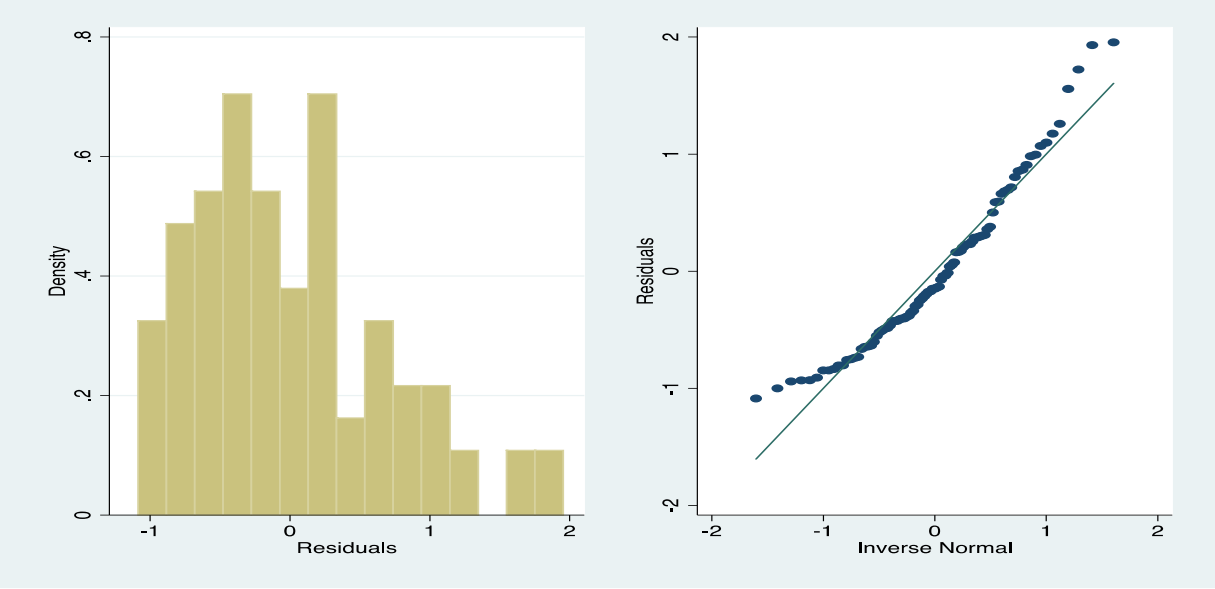
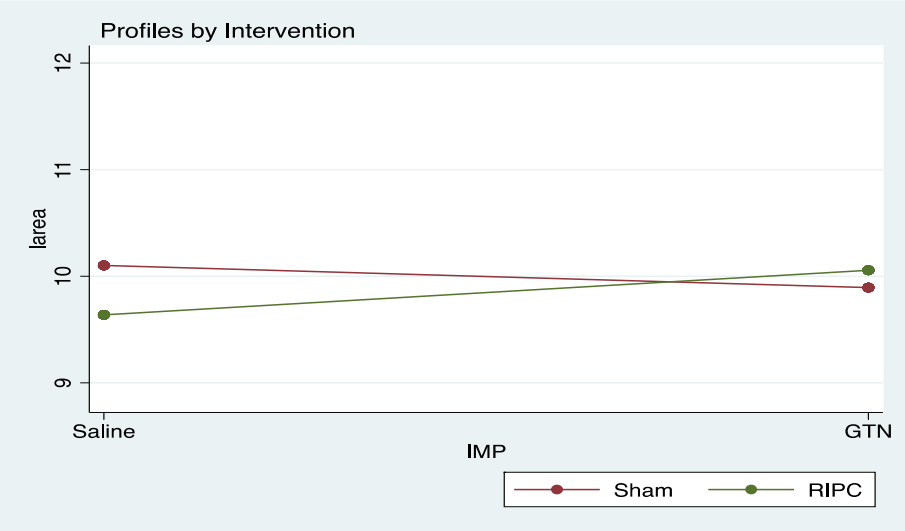
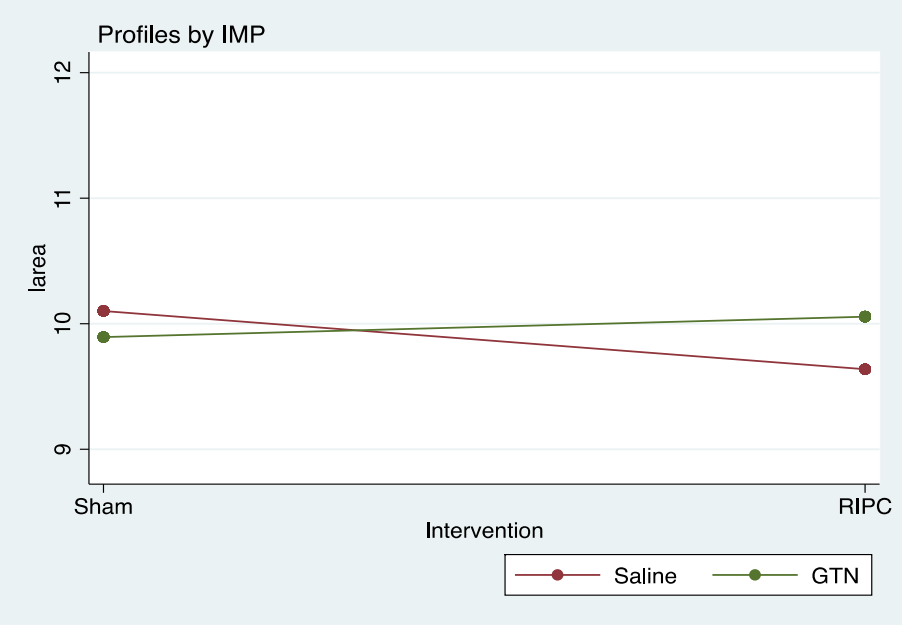


Figure 12. ANOVA regression analysis

The interaction was found to be statistically significant (***F = 4.3, p = 0.0411***), which meant that the effect of RIPC could not be generalised for both patients who received GTN and those who received saline, but for different levels of one treatment, the effect of the other treatment varies. Plot 1 shows that RIPC decreases log (AUC) for those under Saline, while RIPC is associated with a higher AUC when patients are also taking GTN.

<u>Plot 1</u>: RIPC decreases log (AUC) for a patient who received saline, while RIPC is associated with a higher AUC for patients who received GTN.





We opted to use a linear regression model as it was useful to estimate the effects. The model was based on the log-transformed AUC (**larea**). However, in the table below, coefficients have been exponentiated, so they were interpretable as ratios of geometric means. For example, the effect of RIPC was a 37.1% reduction in AUC geometric means for patients only in the RIPC treatment group (group 3), compared with the control group (group 1).

Table 3.4.4.1 Log transformed AUC (larea)/Linear regression model.

larea	Geometric mean ratio	Standard Error	t-test	P	[95% Conf. Interval]	
IMP(GTN)	0.8	0.1	-0.90	0.369	0.5	1.2
Intervention (RIPC)	0.6	0.1	-2.19	0.031	0.4	0.9
IMP/intervention	1.8	0.5	2.07	0.041	1.0	3.4
Constant	24381.6	3877.9	63.51	0.000	17773.3	33446.9

Since interaction was significant, the table above gave only a partial representation of all of the possible contrasts between groups. Pairwise comparisons of marginal linear predictions were used to assess the significance of all possible pair comparisons. Here too, coefficients and confidence intervals were exponentiated, and since the interaction was significant (see above), marginal comparisons of the treatments (top two rows in the table below) were not useful to assess treatments effects.

Table 3.4.4.2 Pairwise comparison of marginal linear prediction with exponentiated coefficients and confidence interval.

	Exponentiated coefficients	Standard Error	t-test	P	[95% Conf. Interval]	
IMP						
GTN vs. Saline	1.1	0.1	0.69	0.490	0.8	1.5
Intervention						
RIPC vs. Sham	0.8	0.1	-0.99	0.323	0.6	1.1
IMP/Intervention						
	Exponentiated coefficients	**Standard Error**	**t-test**	**p**	**[95% Conf. Interval]**	

(Saline/RIPC) vs. (Saline/Sham)	0.6	0.1	-2.19	0.031	0.4	0.9
(GTN/Sham) vs. (Saline/Sham)	0.8	0.1	-0.90	0.369	0.5	1.2
(GTN/RIPC) vs. (Saline/Sham)	0.9	0.2	-0.22	0.829	0.6	1.4
(GTN/Sham) vs. (Saline/RIPC)	1.3	0.2	1.17	0.245	0.8	1.9
(GTN/RIPC) vs. (Saline/RIPC)	1.5	0.3	2.14	0.035	1.0	2.2
(GTN/RIPC) vs. (GTN/Sham)	1.1	0.2	0.75	0.453	0.7	1.8

The estimates for comparisons "(**Saline/RIPC**) vs. (**Saline/Sham**)" and "(**GTN/Sham**) vs. (**Saline/Sham**)" were equal to those in the regression model tables since they did not involve interactions, while the others were calculated taking into account the interaction.

Finally, we performed regression analyses only for log-transformed Troponin T values at 6, 12, 24 and 48 hours, controlling for baseline values.

Table 3.4.4.3 Regression analysis only for log-transformed Troponin T values at 6 hours controlling for baseline values.

- ***6 hours***

hsTnT6H	Geometric mean ratio	Standard Error	t-test	P	[95%Conf. Interval]	
IMP						
GTN	0.8	0.1	-1.44	0.152	0.5	1.0
Intervention						
RIPC	0.6	0.1	-2.64	0.009	0.4	0.9
IMP / Intervention						
GTN/RIPC	1.7	0.3	2.42	0.017	1.1	2.7
hsTnT0H	1.2	0.05	4.72	0.000	1.1	1.3
Constant	445.1	70.8	38.34	0.000	325.0	609.6

	Exponentiated coefficients	Standard Error	t-test	P	[95% Conf. Interval]	
IMP						
GTN vs. Saline	1.0	0.1	0.36	0.717	0.8	1.3
Intervention						
RIPC vs. Sham	0.8	0.1	-1.40	0.163	0.6	1.0
IMP/Intervention						
(Saline/RIPC)vs. (Saline/Sham)	0.6	0.1	-2.64	0.009	0.4	0.9
(GTN/Sham)vs. (Saline/Sham)	0.8	0.1	-1.44	0.152	0.5	1.0
(GTN/RIPC)vs. (Saline/Sham)	0.8	0.1	-0.76	0.446	0.6	1.2
(GTN/Sham)vs. (Saline/RIPC)	1.2	0.2	1.22	0.223	0.8	1.7
(GTN/RIPC)vs. (Saline/RIPC)	1.3	0.2	1.99	0.049	1.0	1.8
(GTN/RIPC)vs. (GTN/Sham)	1.1	0.1	0.71	0.477	0.8	1.5

Table 3.4.4.4 Regression analysis only for log-transformed Troponin T values at 12hours controlling for baseline values.

- 12 hours

hsTnT12H	Geometric mean ratio	Standard Error	t-test	P	[95% Conf. Interval]	
IMP						
GTN	0.8	0.1	-0.94	0.347	0.6	1.1
Intervention						
RIPC	0.6	0.1	-2.71	0.007	0.4	0.8
IMP/Intervention						
GTN/RIPC	1.6	0.3	2.11	0.036	1.0	2.6
hsTnT0H	1.1	0.05	3.53	0.001	1.0	1.3
Constant	407.7	67.5	36.29	0.000	293.9	565.6

	Exponentiated coefficients	Standard Error	t-test	P	[95% Conf. Interval	
IMP						
GTN vs. Saline	1.1	0.1	0.75	0.452	0.8	1.3
Intervention						
RIPC vs. Sham	0.8	0.1	-1.70	0.090	0.6	1.0
IMP/Intervention						
(Saline/RIPC) vs. (Saline/Sham)	0.6	0.1	-2.71	0.007	0.4	0.8
(GTN/Sham) vs. (Saline/Sham)	0.8	0.1	-0.94	0.347	0.6	1.2
(GTN/RIPC) vs. (Saline/Sham)	0.8	0.1	-0.70	0.485	0.6	1.2
(GTN/Sham) vs. (Saline/RIPC)	1.3	0.2	1.73	0.086	0.9	1.8

(GTN/RIPC) vs. (Saline/RIPC)	1.4	0.2	2.07	0.040	1.0	1.9
(GTN/RIPC) vs. (GTN/Sham)	1.0	0.1	0.26	0.798	0.7	1.4

Table 3.4.4.5 Regression analysis only for log-transformed Troponin T values at 24 hours controlling for baseline values

- 24 Hours

hsTnT24H	**Geometric mean ratio**	**Standard Error**	**t-test**	**P**	**[95% Conf. Interval]**	
IMP						
GTN	0.7	0.1	-2.11	0.037	0.5	0.9
Intervention						
RIPC	0.6	0.1	-2.92	0.004	0.4	0.8
IMP/Intervention						
GTN/RIPC	1.7	0.3	2.66	0.009	1.1	2.6
hsTnT0H	1.2	0.05	4.93	0.000	1.1	1.3
Constant	281.7	42.0	37.80	0.000	209.7	378.3

	Exponentiated coefficients	**Standard Error**	**t-test**	**P**	**95% Conf. Interval]**	
IMP						
GTN vs. Saline	0.9	0.1	-0.39	0.694	0.7	1.1
Intervention						
RIPC vs. Sham	0.8	0.1	-1.47	0.144	0.6	1.0
IMP/Intervention						
(Saline/RIPC) vs. (Saline/Sham)	0.6	0.1	-2.92	0.004	0.4	0.8

(GTN/Sham) vs. (Saline/Sham)	0.7	0.1	-2.11	0.037	0.5	0.9
(GTN/RIPC) vs. (Saline/Sham)	0.8	0.1	-1.32	0.189	0.6	1.1
(GTN/Sham) vs. (Saline/RIPC)	1.1	0.1	0.78	0.438	0.8	1.5
(GTN/RIPC) vs. (Saline/RIPC)	1.2	0.1	1.65	0.101	0.9	1.6
(GTN/RIPC) vs. (GTN/Sham)	1.1	0.1	0.81	0.420	0.8	1.5

Table 3.4.4.6 Regression analysis only for log-transformed Troponin T values at 48 hours controlling for baseline values.

- 48 hours

hsTnT48H	**Geometric mean ratio**	**Standard Error**	**t-test**	**P**	**[95% Conf. Interval]**	
IMP						
GTN	0.9	0.1	-0.28	0.776	0.6	1.3
intervention						
RIPC	0.7	0.1	-2.00	0.047	0.5	0.1
IMP/Intervention						
GTN/RIPC	1.3	0.3	1.36	0.177	0.8	2.2
hsTnT0H	1.2	0.06	3.86	0.000	1.1	1.3
Constant	205.4	35.4	30.87	0.000	145.1	289.0

	Exponentiated coefficients	**Standard Error**	**t-test**	**P**	**95% Conf. Interval**	
IMP						
GTN vs. Saline	1.1	0.1	0.94	0.352	0.8	1.4
Intervention						
RIPC vs. Sham	0.8	0.1	-1.47	0.143	0.6	1.0

IMP/Intervention						
(Saline/RIPC vs. (Saline/Sham)	0.7	0.1	-2.00	0.047	0.5	0.1
(GTN/Sham) vs. (Saline/Sham)	0.9	0.1	-0.28	0.776	0.6	1.3
(GTN/RIPC)vs. (Saline/Sham)	0.9	0.1	-0.40	0.692	0.6	1.3
(GTN/Sham) vs. (Saline/RIPC)	1.3	0.2	1.66	0.100	0.9	1.9
(GTN/RIPC)vs. (Saline/RIPC)	1.3	0.2	1.70	0.091	0.9	1.8
(GTN/RIPC) vs. (GTN/Sham)	0.9	0.1	-0.09	0.929	0.7	1.3

The complete case analysis showed that there was **no significant main effect of GTN**. In particular:

- **For AUC**:

  There was a **significant interaction term** (**p = 0.041**). The only significant reduction in AUC was noticed for group 3 (**RIPC+Saline**) vs. group 1 (**Sham+Saline**), which was when comparing groups where ***no GTN treatment was involved.***

- **For Troponin T values at any time point**

  The interaction term was significant at **6, 12 and 24 hours**. At all these time points the contrast **RIPC+Saline** (group 3) VS **Sham+Saline** (group 1) was significant and could be interpreted as the same before. Also, for time points **6 and 12 hours**, the outcome for group 4 (**RIPC+GTN**) was worse than that for group 3 (**RIPC+Sham**), a result that highlighted the role of the interaction term for the two treatments again. Finally, there was no significant effect at **48h** for any treatment.

As the amount of missing data was substantial, it was difficult to base conclusion on complete case analysis. After reviewing the causes of missing data, it was noticed that data were missing completely at random (**MCAR** data were not missing); therefore, the results from complete case analysis would have not been biased. We opted for a multiple imputations strategy, as shown below, and based the conclusion upon those.

3.4.5 Multiple Imputation Analyses

Notes

- In our CRF, the symbol == was used to indicate the number zero. For example, grafts== meant there were no grafts used.
- We adopted the **ERICCA** trial imputation model and created 20 imputed datasets.

The extent of absence of hsTnT values is shown in the table below. As demonstrated, the most absent data belonged to the 48H point.

Table 3.4.5.1 Multiple Imputation analysis

Variable	Missing Observations	Total Observations
hsTnT 0H	20	164
hsTnT 6H	37	147
hsTnT12H	26	158
hsTnT24H	31	153
hsTnT48H	57	127

Average values *for all the 20 imputed datasets* show that AUC shapes are similar to those obtained using complete data (see above).

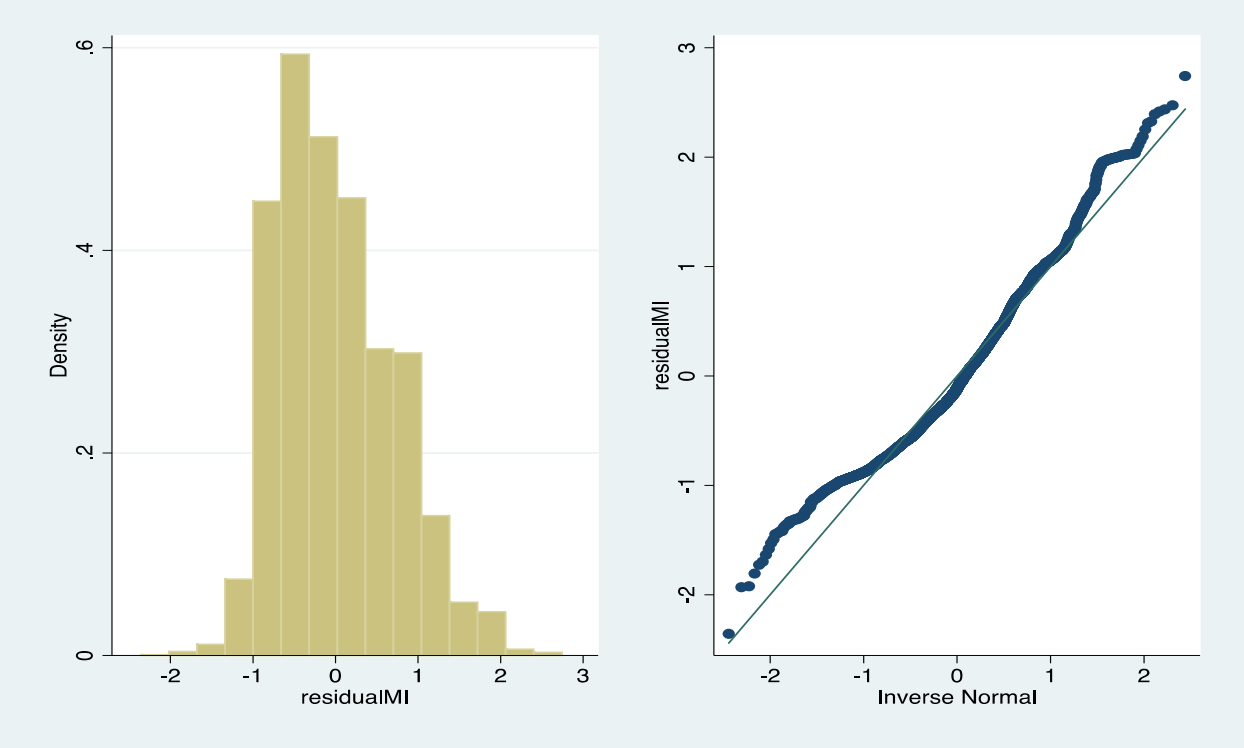
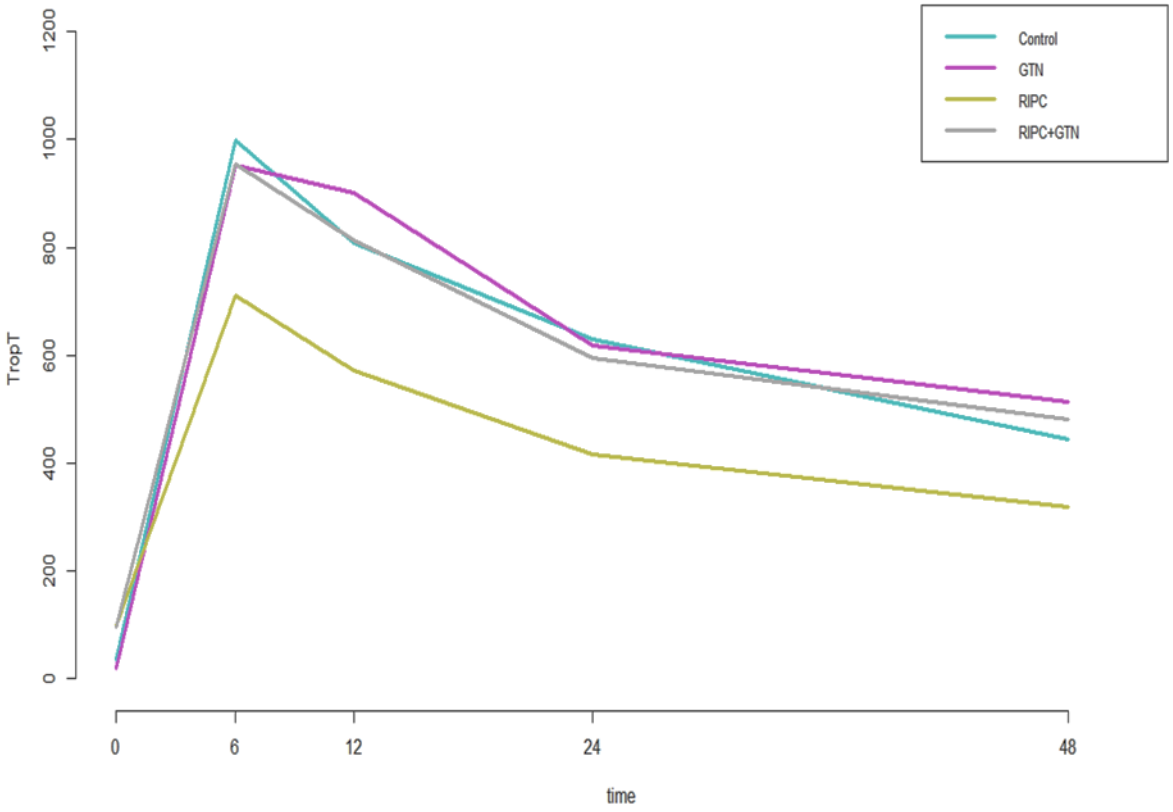


Figure 13. Normality of data is achieved after multiple imputations.



Time in hours / Troponin T level ng/l

Figure 14. The graphical representation of the MEAN areas under the curve using Imputed data.

The results were assessed directly using a regression model and pairwise comparisons. Once again coefficients were exponentiated to be interpretable as ratios of geometric means. None of the coefficients were significant.

Table 3.4.5.2 Imputed data AUC not showing any significant interaction.

larea	Geometric mean ratio	Standard Error	t-test	P	[95% Conf. Interval]	
IMP(GTN)	0.9	0.1	-0.50	0.618	0.6	1.2
Intervention (RIPC)	0.7	0.1	-1.77	0.078	0.5	1.0
IMP/Intervention	1.3	0.3	1.47	0.144	0.9	2.1
Constant	23274.1	2601.6	89.95	0.000	18661.2	29027.3

Pairwise comparisons did not show any significant interactions also.

Table 3.4.5.3 Pairwise comparisons not showing any significant interactions.

	Exponentiated coefficients	Standard Error	[95% Conf. Interval]	
IMP				
GTN vs. Saline	1.0	0.1	0.8	1.3
Intervention				
RIPC vs. Sham	0.8	0.1	0.7	1.1
IMP/Intervention				
(Saline/RIPC)vs.(Saline/Sham)	0.7	0.1	0.5	1.0
(GTN/Sham) vs. (Saline/Sham)	0.9	0.1	0.6	1.2
(GTN/RIPC) vs. (Saline/Sham)	0.9	0.1	0.7	1.3
(GTN/Sham) vs. (Saline/RIPC)	1.2	0.2	0.8	1.6
(GTN/RIPC) vs. (Saline/RIPC)	1.2	0.2	0.9	1.7
(GTN/RIPC) vs. (GTN/Sham)	1.0	0.1	0.7	1.4

We replicated the analysis only looking at single time points, as in the complete data analysis.

Table 3.4.5.4 Replication analysis looking at 6 hours in the complete data analysis.

- 6 hours

hsTnT6H	Geometric mean ratio	Standard Error	t-test	P	[95% Conf. Interval]	
IMP	0.8	0.1	-0.83	0.409	0.6	1.2
Intervention	0.6	0.1	-2.46	0.015	0.4	0.9
IMP/Intervention	1.3	0.3	1.47	0.143	0.9	2.1
hsTnT 0H	1.2	0.05	4.25	0.000	1.1	1.3

Constant	468.9	76.0	37.94	0.000	340.1	646.5

	Exponentiated coefficients	**Standard Error**	**[95% Conf. Interval]**	
IMP				
GTN vs. Saline	1.0	0.1	0.8	1.2
Intervention				
RIPC vs. Sham	0.8	0.1	0.6	0.9
IMP/Intervention				
(Saline/RIPC) vs. (Saline/Sham)	0.6	0.1	0.4	0.9
(GTN/Sham) vs. (Saline/Sham)	0.8	0.1	0.6	1.2
(GTN/RIPC) vs. (Saline/Sham)	0.8	0.1	0.6	1.1
(GTN/Sham) vs. (Saline/RIPC)	1.3	0.2	0.9	1.8
(GTN/RIPC) vs. (Saline/RIPC)	1.2	0.1	0.9	1.6
(GTN/RIPC) vs. (GTN/Sham)	0.9	0.1	0.6	1.2

Table 3.4.5.5 Replication analysis looking at 12 hours in the complete data analysis.

- 12 hours

hsTnT12H	**Geometric mean Ratio**	**Standard Error**	**t-test**	**P**	**[95% Conf. Interval]**	
IMP (GTN)	0.9	0.1	-0.54	0.592	0.6	1.2
Intervention (RIPC)	0.6	0.1	-2.76	0.007	0.4	0.8
IMP/Intervention	1.5	0.3	1.77	0.079	0.9	2.4
hsTnT 0H	1.1	0.05	3.16	0.002	1.0	1.3
Constant	426.9	74.3	34.80	0.000	302.6	602.4

	Exponentiated coefficients	Standard Error	[95% Conf. Interval]	
IMP				
GTN vs. Saline	1.1	0.1	0.8	1.4
Intervention				
RIPC vs. Sham	0.7	0.1	0.6	0.9
IMP/Intervention				
(Saline/RIPC)vs.(Saline/Sham)	0.6	0.1	0.4	0.8
(GTN/Sham)vs. (Saline/Sham)	0.9	0.1	0.6	1.2
(GTN/RIPC)vs. (Saline/Sham)	0.8	0.1	0.6	1.2
(GTN/Sham)vs. (Saline/RIPC)	1.4	0.2	1.0	2.0
(GTN/RIPC) vs. (Saline/RIPC)	1.3	0.2	0.1	1.9
(GTN/RIPC) vs. (GTN/Sham)	0.9	0.1	0.6	1.3

Table 3.4.5.6 Replication analysis looking at 24 hours in the complete data analysis.

- 24 hours

hsTnT24H	Geometric mean ratio	Standard Error	t	P	[95% Conf. Interval]	
IMP	0.9	0.1	-0.61	0.544	0.6	1.2
Intervention	0.6	0.1	-2.60	0.010	0.4	0.9
IMP/Intervention	1.5	0.3	1.89	0.061	0.9	2.3
hsTnT 0H	1.2	0.05	4.21	0.000	1.1	1.3
Constant	269.0	45.7	32.94	0.000	192.0	377.0

	Exponentiated coefficients	Standard Error	[95% Conf. Interval]	
IMP				
GTN vs. Saline	1.1	0.1	0.8	1.4
Intervention				
RIPC vs. Sham	0.8	0.1	0.6	1.0
IMP/Intervention				
(Saline/RIPC)vs. (Saline/Sham)	0.6	0.1	0.4	0.9
(GTN/Sham)vs. (Saline/Sham)	0.9	0.1	0.6	1.2
(GTN/RIPC)vs. (Saline/Sham)	0.9	0.1	0.6	1.2
(GTN/Sham)vs. (Saline/RIPC)	1.3	0.2	0.9	1.9
(GTN/RIPC)vs. (Saline/RIPC)	1.3	0.2	1.0	1.8
(GTN/RIPC) vs. (GTN/Sham)	0.1	0.1	0.7	1.3

Table 3.4.5.7 Replication analysis looking at 48 hours in the complete data analysis.

- 48 hours

hsTnT 48H	Geometric mean ratio	Standard Error	t	P	[95% Conf. Interval]	
Imp	1.0	0.1	0.13	0.899	0.7	1.4
Intervention	0.6	0.1	-2.36	0.020	0.5	0.9
IMP/Intervention	1.4	0.3	1.59	0.113	0.9	2.1
hsTnT 0H	1.2	0.05	3.92	0.000	1.1	1.3
Constant	205.5	34.2	31.95	0.000	147.6	286.1

	Exponentiated coefficients	Standard Error	[95% Conf. Interval]	
IMP				
GTN vs. Saline	1.2	0.1	0.9	1.5
Intervention				
RIPC vs. Sham	0.8	0.1	0.6	1.0
IMP/Intervention				
(Saline/RIPC)vs. (Saline/Sham)	0.6	0.1	0.5	0.9
(GTN/Sham)vs. (Saline/Sham)	1.0	0.1	0.7	1.4
(GTN/RIPC)vs. (Saline/Sham)	0.1	0.1	0.7	1.3
(GTN/Sham)vs. (Saline/RIPC)	1.4	0.2	1.0	2.0
(GTN/RIPC)vs. (Saline/RIPC)	1.4	0.2	1.0	1.9
(GTN/RIPC) vs. (GTN/Sham)	0.9	0.1	0.7	1.3

Comments on Multiple Imputation analysis

The complete case data showed that there was no significant reduction in any time-point or AUC troponin with GTN versus no GTN. In more detail:

- **Considering AUC**:

  There was no main effect for RIPC, nor for GTN nor the interaction term. Even if not significant, the average effect of GTN alone was to ***increase*** AUC.

- **Considering Troponin T values at any time point**:

  RIPC has an overall effect at 6h, given that the interaction term was not significant. The same happened at 12h. In both cases, the reduction of the

troponin T (geometric) mean value was slightly above 20%. No significant effect was detected at both 24 and 48 hours.

3.5 Discussion

3.5.1 Effects of simultaneous multi-limb RIPC on PMI

Following cardiac surgery, the release of cardiac enzymes TnT has been associated with worse short- and long-term clinical outcomes (38, 39, 59) and therefore has a significant impact on the patient's outcome.

As previously discussed, one of the underlying causes of PMI during cardiac surgery is acute IRI secondary to intermittent aortic cross-clamp, ICCF or intermittent or continuous administration of cardioplegia (41). In this regard, RIPC offers a safe, non-invasive strategy that is capable of reducing PMI in patients undergoing cardiac surgery and therefore potentially improving their outcome.

Przyklenk and colleagues first introduced the concept of RIPC (114), finding a significant reduction in MI size in dogs subjected to four-5-minutes cycles in the LCx area, prior to 1 hour of sustained LAD ischaemia. However, Birnbaum et al. demonstrated that the same strategy could be replicated in a "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 of a rabbit (118). Finally, it was Kharbanda et al. (158) who applied RIPC in healthy volunteers by inducing transient non-invasive limb ischaemia with a simple BP cuff applied to one arm and demonstrating improved endothelial function in the contralateral arm.

Our research group was the first to demonstrate that RIPC reduces PMI in adult patients undergoing cardiac surgery (162). In this trial, 57 patients who were going for

elective CABG with either cardioplegia or ICCF, were randomised to into 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). The results showed a 43% reduction in those patients who underwent RIPC release over the 72-hour peri-operative period compared to controls. Further trials confirmed the results in 45 non-diabetic patients undergoing elective CABG with or without valve surgery and receiving cold-blood cardioplegia alone (190). In this trial, RIPC reduced the 72h troponin area under the curve by 42.4% concluding that RIPC can reduce PMI irrespective of the of myocardial preservation technique.

The concept of RIPC was extended to patients receiving antegrade cold crystalloid cardioplegia in a study by Thielmann et al. (191), where non-diabetic patients undergoing three-vessel disease CABG sustained a 44.5% reduction in AUC cTnI, showing that RIPC can extend protection to the patient undergoing CABG receiving crystalloid cardioplegia. In another trial conducted by Thielmann et al. (171), using the same preconditioning stimulus, they showed that the ratio of RIPC/control for cTnI AUC was 0.83. Interestingly, it demonstrated a significant reduction in the combined endpoint of all-cause mortality, major adverse cardiac and cerebrovascular events, and repeat revascularisation.

However, several other studies showed a limited benefit of RIPC in the setting of cardiac surgery. For example, Wagner et al. (192) applied a RIPC stimulus 18 hours before surgery (CABG±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. Interestingly, a third treatment group where patients received tramadol 200 mg at 19:00 hours the day before surgery and at 06:00 hours on the day of the operation, PMI was significantly

higher than in the control group at all time-points. This would have indicated that medication given postoperatively, but within the effect window of RIPC, could have a significant effect on the end-product of preconditioning. Lomivorotov et al. (193) came up with exciting results with regards to RIPC and patients undergoing CABG surgery with cold crystalloid cardioplegia. Importantly, the trial measured cTnI and CK-MB pre-operatively and at 6, 24 and 48 hours post-surgery only, therefore not reflecting the true absolute post-operative release of cardiac biomarkers provided by total AUC.

Rahman et al. (168) investigated patients undergoing isolated first time multi-vessel CABG on cardiopulmonary bypass (CPB) in a single-centre, double-blind, prospective, randomised, placebo-controlled trial. Overall, 162 patients were recruited between January 2007 and March 2009 and randomly assigned on a 1:1 basis; the RIPC stimulus comprised 3 × 5-minute cycles of upper-limb 9-cm cuff inflation to 200 mm Hg separated by 5-minute periods of cuff deflation. The primary endpoint was 48-hour AUC cTnT release, while the secondary endpoints were 6-hour and peak cTnT, ECG changes, cardiac index, inotrope and vasoconstrictor use, renal dysfunction, and lung injury. In contrast to prior smaller studies, RIPC did not reduce troponin release, improve haemodynamics, or enhance renal or lung protection. The study included both elective and urgent patients (post-ACS) in contrast to other studies which recruited elective CABG patients only. More importantly, many of the recruited patients received intra-operative GTN which could have interfered with the cardioprotection provided with RIPC.

High-risk procedures also might affect RIPC. Young et al. (194) failed to demonstrate that a standard preconditioning stimulus could improve PMI, AKI incidence or inotrope requirement in their trial where they recruited 96 patients undergoing high-risk cardiac

surgery, including combined CABG and valve surgery, CABG surgery with LVEF<50%, “redo-operation”, MV surgery, and double or triple valve surgery.

Karuppasamy et al. (195) also failed to demonstrate the benefits of standard RIPC stimulus in their trial, where they recruited 54 patients undergoing elective CABG surgery, and receiving the volatile anaesthetic isoflurane before CPB and the intravenous anaesthetic propofol thereafter until the completion of surgery. Patients subjected to the RIPC stimulus did not show a significant reduction in cTnI, BNP, CK-MB, cytokines and growth factors.

3.5.2 The effect of anaesthetic regime used in cardiac surgery

The interaction between RIPC and Propofol was investigated in the RIPHeart trial (196). A double-blind, multicentre, randomised, controlled trial involving adults who were scheduled for elective cardiac surgery requiring cardiopulmonary bypass under total anaesthesia with intravenous propofol. A total of 1385 patients' data were analysed, of which 692 were in the RIPC group and 693 were in the sham-RIPC group. The results showed no significant differences between the RIPC group and the sham-RIPC group in the level of troponin release, and the trial team came to the conclusion that upper-limb RIPC performed while patients were under propofol-induced anaesthesia did not show a vital benefit among patients undergoing elective cardiac surgery.

The use of anaesthetic regime proved to have a significant effect on RIPC. In two crucial studies (170, 197), the two research teams failed to show significant benefits of RIPC on PMI. In the first trial (170), 72 non-diabetic patients were referred for elective CABG, with crystalloid cardioplegia and induction was achieved with a combination of sufentanil, etomidate and rocuronium. Maintenance was ensured

through either inhaled isoflurane, or propofol infusion with additional sufentanil administered in both cases at the discretion of the anaesthetist. The authors found that isoflurane and RIPC significantly reduced PMI compared to isoflurane alone, whereas no significant difference was found in patients receiving RIPC with propofol, compared to those receiving propofol alone. Lucchinetti et al. (197) used propofol, an opioid (either fentanyl, sufentanil or romifentanil) and rocuronium for induction in their trial, and maintained anaesthesia with isoflurane: RIPC consisted of four 5-minute cycles of 300mmHg cuff inflation/deflation of a BP cuff around the upper leg prior to aortic cross-clamping. The trial did not reduce the total release of cTnT or pro-BNP. Crucially, the incidence of post-operative new arrhythmias and MI was significantly higher in the preconditioned group.

In the ERIC-GTN trial, 158 of the 182 recruited patients received propofol intraoperatively (there were missing data for 12 patients), which accounts for 86.8% of these patients; in contrast, 24 recruited patients did not receive propofol, comprising 13.18% of these patients. Interestingly, it seemed that the RIPC effect was not hindered by propofol, as demonstrated by the RIPHeart trial outcome. It is worth mentioning, however, that RIPC was induced by four cycles of upper-limb ischaemia (5-minute blood-pressure cuff inflation to ≥200 mm Hg, but at least 15 mm Hg higher than the patient's actual systolic arterial pressure, followed by 5-minute cuff deflation) in the RIPHeart trial, which is in contrast to the ERIC-GTN, where RIPC was induced by four cycles of upper and lower limb ischaemia (5-minute blood pressure cuff inflation to 200mmHg, followed by 5-minute cuff deflation).

3.5.3 ERICCA Trial

The ERICCA trial (198) from our institution was a cornerstone trial in the effort to translate the RIPC effect into the clinical setting. A total of 1612 patients (811 in the

control group and 801 in the RIPC group), undergoing elective on-pump CABG with or without valve surgery in 30 cardiac surgery centres in the United Kingdom, and only higher risk patients with EuroScores greater than 5 were recruited. The results showed there were no significant differences between groups in either adverse events, or the secondary endpoints of perioperative myocardial injury (area under the curve for the high-sensitivity assay of serum troponin T at 72 hours), inotrope score (calculated from the maximum dose of the individual inotropic agents administered in the first 3 days after surgery), acute kidney injury, duration of stay in the intensive care unit and hospital, distance on the 6-minute walk test, and quality of life. It is important to mention that 88.5% of the patients recruited to the ERICCA trial received propofol during their procedures.

In a recent meta-analysis (199) of all studies undergoing RIPC for cardiac surgery with cardiopulmonary bypass, these inconsistent findings were all echoed with intervention failing to show a reduced incidence of all-cause mortality, myocardial infarction, stroke, and lengths of ITU and hospital stay. Three studies randomised patients undergoing cardiac surgery to RIPC or sham procedure in the absence of propofol anaesthesia. In this subgroup of 434 randomised patients, 71 of 217 patients (32.7%) who underwent RIPC developed AKI compared with 103 of 217 patients (47.5%) treated with a sham procedure. In this cohort, RIPC resulted in a significant reduction in AKI (RR, 0.700; 95% CI, 0.527-0.930 [P=0.014]). In studies of patients who received propofol anaesthesia, 445 of 1874 (23.7%) patients randomised to RIPC developed AKI compared with 474 of 1901 (24.9%) who underwent a sham procedure. The RR for AKI was 0.928 (95% CI, 0.781-1.102; p=0.39) for RIPC versus sham. This suggests that propofol may mitigate the protective benefits of RIPC.

The neutral results of the ERRICA trial have been widely discussed. Some of the explanation offered was the fact that many of the recruited patients had diabetes. It is believed that individuals with diabetes are less well protected by cardioprotective strategies than non-diabetics, as described in Chapter one, and one quarter of patients recruited into the ERRICA trial were diabetics; many were also on concomitant medications including volatile anaesthetics (40.0%), propofol (88.5%), IV nitrates (28.7%), fentanyl (81.8%), and morphine (29.6%). The mechanism of injury during cardiac surgery includes not only reperfusion injury but microembolisation and inflammation, as the patient is put on and off bypass. The profile of the recruited patient includes many co-morbidities, so it is possible that most of them have been suffering from angina attacks for some time prior to their operation. It is well-described that patients experiencing stable angina for up to three months before acute myocardial infarction have a reduced infarct size (200).

A recent intriguing retrospective analysis of the ERRICA trial database examining the clinical outcomes between those with and without intra-operative IV GTN, demonstrated a "protective effect" of intraoperative GTN which, in principle, is not aligned with the results obtained from the ERIC-GTN trial.

Table 3.5.3.1 Retrospective analysis of the ERRICA trial database examining the clinical outcomes between patients with and without intra-operative IV GTN.

	No intra-operative IV GTN (n=1084)	**Intra-operative IV GTN (n=463)**	**P**
Peri-operative MI	260/1084 (24.0%)	92/463 (19.9%)	0.043*
1-year all-cause mortality	**76/1084 (7.0%)**	**41/463 (8.9%)**	**0.13**

1-year cardiovascular mortality	49/1084 (4.5%)	22/463 (4.8%)	0.47
Stroke	22/1084 (2.0%)	7/463 (1.5%)	0.32
Revascularisation	3/1084 (0.3%)	3/463 (0.6%)	0.25
Length of stay in ITU (days)	3 (1-5)	3 (1-5)	0.08
Length of stay in hospital (days)	10 (7-17)	10 (7-16)	0.81
AKI	413/1038 (39.8%)	152/450 (33.8%)	0.016*
AUC for troponin T (ng.hr/ml), n=718	34.5 (22.4-57.2) n=474	31.2(20.7-53.8) n=244	0.076
Imputed AUC for AUC for troponin T (ng.hr/ml), n=1515	35.3 (22.8-52.6)	36.1 (23.7-57.2)	0.165

Another comparison from the same analysis showed the same “protective effect” of GTN between those with and without baseline oral nitrates or IV GTN.

Table 3.5.3.2 Comparison showing the clinical outcomes between those with and without baseline oral nitrates or IV GTN.

	No intra-operative IV GTN or baseline oral nitrates **(n=927)**	**Intra-operative IV GTN and/ or baseline oral nitrates** **(n=620)**	**P**
Peri-operative MI	230/927 (24.8%)	122/620 (19.7%)	0.010*
1-year all-cause mortality	**63/927 (6.8%)**	**54/620 (8.7%)**	**0.098**
1-year cardiovascular mortality	41/927 (4.4%)	30/620 (4.8%)	0.40
Stroke	18/927 (1.9%)	11/620 (1.8%)	0.49
Revascularisation	2/927 (0.2%)	4/620 (0.6%)	0.18
Length of stay in ITU (days)	3 (1-5)	3 (1-5)	0.38

Length of stay in hospital (days)	10 (8-17)	10 (7-16)	0.14
AKI	355/887 (40.0%)	210/601 (34.9%)	0.027*
AUC for troponin T (ng.hr/ml), n=718	35.0 (22.3-58.9)	31.3 (20.9-50.4)	0.03*
Imputed AUC for AUC for troponin T (ng.hr/ml), n=1515	34.9 (22.6-52.5) n=909	36.6 (22.7-57.2) n=606	0.029*

The next table shows the clinical outcomes divided into 4 groups depending on whether IV GTN or RIC administered. The table demonstrates a reduction in peri-operative MI in patients who have received RIC, GTN or both compared to patients who have not received either.

Table 3.5.3.3 The clinical outcomes divided into four groups depending on whether IV GTN or RIC administered.

	Group 1	**Group 2**	**Group 3**	**Group 4**
	No RIC or IV GTN **(n=503)**	**RIC only** **(n=510)**	**IV GTN only** **(n=226)**	**Both RIC and IV GTN** **(n=223)**
Peri-operative MI	132/503 (26.2%)	115/510 (22.5%)	42/226 (18.6%)	50/223 (22.4%)
P				
Group **1** vs. Group **2**				0.19
Group **1** vs. Group **3**				0.025*
Group **1** vs. Group **4**				0.31

Interestingly, Table 4.1.4 showed that the use of baseline nitrates and/or intraoperative IV GTN in combination with RIC would have an adverse effect on the peri-operative MI, 1-year all-cause mortality and 1-year cardiovascular mortality.

Table 3.5.3.4 Clinical outcomes between the groups that received RIC or sham, stratified by those with or without baseline oral nitrate or intra-operative IV GTN.

Clinical Outcome	Sham	RIC	P value
No baseline oral nitrates or intra-operative IV GTN (n=864)			
Peri-operative MI	119/429 (27.7%)	98/435 (22.5%)	0.046*
1-year all-cause mortality	**32/429 (7.5%)**	**30/435 (6.9%)**	**0.43**
1-year cardiovascular mortality	20/429 (4.7%)	21/435 (4.8%)	0.52
Stroke	7/429 (1.6%)	10/435 (2.3%)	0.32
Revascularisation	2/429 (0.5%)	0/435 (0%)	0.25
AKI	162/418 (38.8%)	170/423 (40.2%)	0.36
AUC for troponin T (ng.hr/ml), n=412	37.3 (23.1-61.6) n=201	31.1 (20.9-55.3) n=211	0.052
Imputed AUC for AUC for troponin T (ng.hr/ml), n=846	35.2 (24.1-54.6) n=418	35.6 (21.6-51.6) n=428	0.25
Baseline oral nitrates and/ or intra-operative IV GTN (n=598)			
Peri-operative MI	55/300 (18.3%)	67/298 (22.5%)	0.12
1-year all-cause mortality	**14/300 (4.7%)**	**36/298 (12.1%)**	**<0.001***
1-year cardiovascular mortality	6/300 (2.0%)	23/298 (7.7%)	<0.001*
Stroke	5/300 (1.7%)	5/298 (1.7%)	0.62
Revascularisation	2/300 (0.7%)	2/298 (0.7%)	0.69
AKI	104/299 (34.8%)	105/287 (36.6%)	0.36
AUC for troponin T (ng.hr/ml), n=298	33.1 (22.2-50.7) n=153	28.7 (20.0-50.0) n=145	0.28

Imputed AUC for AUC for troponin T (ng.hr/ml), n=584	36.9 (25.3-59.4) n=291	36.5 (22.8-56.9) n=293	0.17
Baseline oral nitrates only (n=149)			
1-year all-cause mortality	**1/74 (1.4%)**	**9/75 (12.0%)**	**0.018**
1-year cardiovascular mortality	1/74 (1.4%)	7/75 (9.3%)	0.063
IV GTN only (n=374)			
1-year all-cause mortality	**9/192 (4.7%)**	**18/182 (9.9%)**	**0.052**
1-year cardiovascular mortality	3/192 (1.6%)	10/182 (5.5%)	0.048
Both oral nitrates and IV GTN (n=75)			
1-year all-cause mortality	**4/34 (11.8%)**	**9/41 (22.0%)**	**0.25**
1-year cardiovascular mortality	2/34 (5.9%)	6/41 (14.6%)	0.28

From this retrospective analysis of the ERICCA trial, IV GTN and/or oral nitrates appear to reduce PMI incidence and AUC troponin, while RIPC appears to reduce PMI incidence but not in the presence of GTN and/or nitrates, and when given in combination, there appears to be a worsening mortality.

In ERIC-GTN, the initial trial protocol stated that the follow-up process for recruited patients would end in hospital discharge. However, and based on the above ad-hoc analysis of the ERICCA, we obtained the necessary approvals to extend the follow-up period to one year. The results showed that although there was a potential negative interaction between RIC and GTN in terms of one-year all-cause mortality from the post-hoc analysis of ERICCA trial, there was no evidence of mortality increase from the current ERIC-GTN trial, but the number of events were low.

3.5.3.5 ERIC-GTN patient recruited at UCLH NHS Trust and their distribution among the trials' four arms.

Study Arms	Sham/Saline	Sham/GTN	RIPC/Saline	RIPC/GTN
Number of Patients recruited	31	32	28	34
Number of Deceased Patients	0	1	2	4
Missing outcomes	1	2	5	1
Percentage of deceased per arm	0%	3.125%	7.142%	11.764%

Total of missing data in this site	16
Total known randomisation but missing outcome	9
Total missing randomisation information	7

3.5.3.6 ERIC-GTN patient recruited at BARTS' NHS Trust and their distribution among the trials' four arms.

Study Arms	Sham/Saline	Sham/GTN	RIPC/Saline	RIPC/GTN
Number of Patients recruited	14	16	14	15
Number of Deceased Patients	0	2	0	0
Missing outcomes	0	1	2	0
Percentage of deceased per arm	0%	12.5%	0%	0%

Total of missing data in this site	**5**
Total known randomisation but missing outcome	**3**
Total missing randomisation information	**2**

3.5.4 The Effect of Diabetes

The fact that many CAD patients suffer from a concomitant medical condition, especially diabetes, can have a significant influence on the cardioprotective effect of RIPC. Animal studies have shown that the diabetic myocardium may have an increased resistance to IRI compared to the non-diabetic heart, although different results have been obtained from different animal models (201, 202). 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. Most of these trials also suggested reduced resistance to IRI in the long-term DM (203, 204). In animal models, IPC has been shown to reduce IRI in DM type 1. However, interestingly, this effect was lessened in rats which had been diabetic for a few weeks (205). In the first animal models with DM type 2, the authors showed that animals with chronic diabetes sustained less IRI, but failed to demonstrate reduced myocardial injury by IPC in these models (202).

Human studies of the cardioprotective effects of IPC in diabetes have mainly focused on analysis of the recovery of atrial trabecular contractility in response to an IPC stimulus. The Hatter Cardiovascular Institute demonstrated that a more significant contractile function was recovered in human atrial trabeculae isolated from diabetic patients undergoing CABG surgery, when subjected to prolonged/more severe

hypoxic preconditioning (206). In this study the atrial trabeculae were isolated and subjected to 90 minutes of hypoxia followed by 120 minutes of re-oxygenation, following which the percentage recovery of baseline contractile function was determined. The atrial trabeculae were randomised to control groups; standard hypoxic preconditioning (HPC) compromised of 4 minutes of hypoxia/16 minutes of re-oxygenation before the 90 minute index hypoxic period, while the prolonged HPC protocol was 7 minutes of hypoxia/16 minutes of re-oxygenation before the index hypoxic period. Also, basal levels of Akt phosphorylation were determined in right atrial appendages harvested from non-diabetic and diabetic patients.

The study showed that standard HPC improved baseline contractile function in human atrial trabeculae harvested from non-diabetic patients, but not in atrial trabeculae isolated from diabetic patients. However, the prolonged HPC protocol did improve the baseline contractile function in atrial trabeculae harvested from diabetic patients. Western blot analysis demonstrated lower levels of the prosurvival kinase, phosphorylated Akt, in the diabetic myocardium compared to the non-diabetic myocardium. The trial team concluded that the threshold for preconditioning the diabetic myocardium is elevated, which may be related to down-regulation of the PI3K-Akt pathway.

CHAPTER 4

4.1 Summary and Future Considerations

As discussed in Chapter one, CAD is the leading cause of death worldwide, and any effort to reduce the mortality and morbidity caused by CAD would be welcomed. The complete data set analysis of the ERIC-GTN trial showed that the effect of RIPC is a 37.1% reduction in AUC geometric mean (p=0.031), which is significant for the time points 6, 12 and 24 hours for patients only in the RIPC treatment group (group 3), compared with the control group (group 1). However, the only significant reduction in AUC was noticed for group 3 (**RIPC+Saline**) vs. group 1 (**Sham+Saline**), which involved comparing groups where no GTN treatment was involved. The complete data analysis also showed that the effect of RIPC could not be generalised for patients who received GTN, and patients who received normal Saline. RIPC has decreased log (AUC) for patients who received normal Saline, while RIPC was associated with a higher AUC when patients received GTN. The interaction was statistically significant (F=4.3, p=.0411).

In Bart's NHS Trust, for example, most patients undergoing CABG would routinely receive GTN infusion intraoperatively. Interestingly, this was also noticed in a clinical setting, that 86.81% of the patients recruited to the ERIC-GTN trial received propofol intraoperatively, as it was believed that propofol could abrogate the protective effect of RIPC (196).

The ERIC-GTN trial has provided an insight into the interaction between RIPC and GTN. As demonstrated in the ERIC-GTN data analysis and from the retrospective data analysis from the ERICCA trial, it seems that GTN have a negative interaction with RIPC.

It is clear from the previous retrospective analysis that further in-depth trials are needed to clarify the relationship between GTN and RIPC in a clinical setting, as the patients involved could also be long-term sufferers of DM and also receiving a cocktail of drugs to manage their risk factors.

Patients who undergo CABG as a revascularisation method usually have a background of DM and other CV risk factors. As demonstrated before, DM has an attenuating effect of RIPC in the long-term diabetic myocardium, which could negatively affect the protective effect of RIPC.

Due to these inconsistent results in cardiac surgery and essentially stable patients, RIPC has been used to explore whether it can reduce cardiac and renal events in patients that are unstable. The rationale for this is that the magnitude of ischaemia in stable patients is not significant enough for RIPC to show a beneficial effect; therefore, current studies are investigating whether acute ST-elevation myocardial infarction patients may be the correct subgroup. The Remote Ischaemic Conditioning Reduces Myocardial Infarct Size in STEMI Patients Treated by Thrombolysis (ERIC-LYSIS) trial (207) recruited 519 STEMI patients who were randomly assigned to receive either RIC (n = 258) or the control protocol (n = 261). The two groups had similar patient characteristics. AUC troponin T data were available for 414 patients, and AUC CK-MB data were available for 407 patients. Median enzymatic MI size was 32% smaller by 24-h AUC troponin T and 19% smaller by 24-h AUC CK-MB in patients who received RIC compared with control patients. The trial team concluded that RIC reduced MI size in STEMI patients treated with thrombolysis, making this non-invasive, easily applied, low-cost therapy an attractive option in developing nations where health care resources are limited, and the current therapy is not optimal.

The CONDI trial (208) seemed to support the rhetoric that RIPC benefits would be more prominent in unstable patients. In this trial, 333 patients with a suspected first acute ST-elevation myocardial infarction were randomised to receive primary percutaneous coronary intervention with (n = 166) or without (n = 167) remote ischaemic conditioning. Patients received remote conditioning during transport to the hospital, and primary percutaneous coronary intervention in the hospital. The primary endpoint was myocardial salvage index at 30 days after primary percutaneous coronary intervention, measured by myocardial perfusion imaging as the proportion of the area at risk salvaged by treatment. The median salvage index was 0.75 (IQR 0.50-0.93, n=73) in the remote conditioning group versus 0.55 (0.35-0.88, n=69) in the control group, with a median difference of 0.10 (95% CI 0.01-0.22; p=0.0333); the mean salvage index was 0.69 (SD 0.27) versus 0.57 (0.26), with a mean difference of 0.12 (95% CI 0.01-0.21; p=0.0333). This trial indicated that remote ischaemic conditioning before hospital admission increases myocardial salvage and has a favourable safety profile. The trial team recommended larger trials to establish these findings.

The follow-up trial CONDI2/ERIC-PPCI (209) could add to our understanding of the effect of RIPC in a clinical setting and will determine whether RIC can improve long-term clinical outcomes in STEMI patients undergoing PPCI. The CONDI2/ERIC-PPCI trial is a European multicentre (Denmark, Serbia, Spain, and United Kingdom) randomised controlled clinical trial of 5400 STEMI patients undergoing PPCI. Eligible patients will be randomised to receive either RIC (four-5 min inflations/deflations of a cuff placed on upper arm) or control before PPCI. The primary endpoint of the study will be cardiac death and heart failure hospitalisation. Secondary endpoints will include rates of all-cause death, coronary revascularisation, re-infarction, stroke at 30 days

and 12 months, TIMI flow post-PPCI, ST-segment resolution on ECG, and quality of life. MI size will be determined in cardiac enzyme and cardiac MRI sub-studies.

4.2 Limitations

The limitations of the ERIC-GTN trial included lack of standardisation of pre and peri-operative medication which, as explained above, could interfere with the effect of RIPC. In addition, many recruited patients suffered from chronic conditions such as DM which, in turn, could influence RIPC effect.

In order to increase recruitment and due to competing trials, we recruited patients who underwent a variety of procedures including bypass and/or valves (replace or repair) operations. This would have involved a host of surgical techniques and different arrest protocols, which in turn affected troponin levels.

In our data analysis and to investigate its effect of the results, we used multiple imputations. For unbiased results, this approach required the assumption that data are missing completely at random, which was the case in the ERIC-GTN trial.

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Patient Information Sheet Version 5.1 01/04/2018

Part 1

Study title: The effect of remote ischaemic preconditioning (RIPC) and glyceryl trinitrate (GTN) on peri-operative myocardial injury in cardiac bypass surgery patients (ERIC-GTN study)

Sub Study 1: Examining the effects of RIPC and GTN on left ventricular function post operatively

Sub Study 2: Investigating cardioprotective biomarkers

Protocol Reference Number:

Investigation into the role of GTN & RIPC in cardiac surgery – protocol reference number 120541

We would like to invite you to take part in our research study and sub-study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. We'd suggest this should take about 20 minutes. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?

This study examines a new concept where the blood supply to the forearm and leg stopped for a short time. Two blood pressure cuffs are inflated one on the arm and one on the leg. This is called remote ischaemic pre-conditioning (RIPC). RIPC may protect the heart during heart surgery.

We also wish to examine the effect of a drug that is used sometimes during heart surgery, called Glyceryl Trinitrate (GTN). We think GTN may influence the level of protection the heart receives from RIPC. This study hopes to look at the effect of using RIPC and GTN.

In one of the sub-studies will look at you heart function a few weeks to months after your operation. We wish to see if GTN and RIPC improves your heart function.

Sub-study 1 will look at your heart function a few weeks to months after your operation. We wish to see if GTN and RIPC improves your heart function.Sub-study 2 will look into the relationship between (RIPC) and other markers in your blood which could help protect your heart muscle.

2. Why have I been invited?

You have been admitted to the hospital for heart surgery. We are looking for patients having heart surgery that meet certain criteria and may benefit from this treatment. 260 patients will be recruited in total.

3. Do I have to take part?

It is up to you to decide to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time without giving a reason. This would not affect the standard of care you receive.

4. What will happen to me if I take part?

You will be involved in this research for the duration of your hospital stay. We will also follow your clinical outcome, up to one year after your discharge from hospital. We anticipate that the research study will last for 2 years. If you return to the hospital as part of your routine follow up, you may have an ultrasound scan of your heart. As part of the sub-study, we ask your permission to collect this data.

Randomised Trial – Sometimes we don‘t know which way of treating patients is best. To find out, we need to compare different treatments. We put people into groups and give each group a different treatment. The results are compared to see if one is better. To try to make sure the groups are the same to start with, each patient is put into a group by chance (randomly).

You will be randomly assigned to one of 4 treatment groups.

Group 1: No RIPC and no GTN.

Group 2: RIPC with no GTN

Group 3: No RIPC with GTN

Group 4: RIPC with GTN.

We will not know which group you will be allocated to in advance.

Your anaesthetic and surgery will proceed as normal. However, the group to which you are allocated will be coded and the code can be broken in an emergency. **After you are asleep with the general anaesthetic**, we will take a blood sample from you. You will then receive the treatment have been assigned.

We have defined below what each term means:

a) RIPC: A blood pressure cuff will be placed around one arm and one thigh. Both cuffs with be inflated to above your blood pressure and kept there for five minutes. These will then be deflated for five minutes and the same cycle will be repeated three times.
b) No RIPC: A blood pressure cuff will be placed around one arm and one thigh. There will be no inflation of either cuff. They will be kept on for 30 minutes.
c) GTN: An infusion of glyceryl trinitrate (GTN) will be started after you are taken to the operating theatre. The anaesthetist will monitor your heart using ECG (sticky dots on the skin) and blood pressure recordings.
d) No GTN: An infusion of salt water (normal saline) will be started after you are in the operating theatre. The anaesthetist will monitor your heart using ECG and blood pressure recordings.

Your operation will proceed as explained by your surgeon and will be the same whether or not you choose to take part or not. The infusion of GTN/normal saline will be stopped after you come off the heart bypass machine. The rest of the surgery will continue as planned. After your operation, you will be taken to the intensive care unit for routine post-operative care. We will visit you during this time to take blood samples 12 hours after, on day 1, and day 2. Often these tests can be taken with your routine blood tests, so does not involve any extra needle sticks. In total, 6 blood samples will be taken from you. Additionally we will gather some information about how you are doing (up to one year) to analyse the effect of the intervention you received

Blind trial – In a ‘blind trial' you will not know which treatment group you are in. If the trial is a ’double blind trial‘, neither you nor your doctor will know in which treatment group you are (although, if your doctor needs to find out he/she can do so). In this trial, you, your anaesthetist, your surgeon and the trial statistician who analyses the data will be blinded.

Placebo – A placebo is a dummy treatment such as salty water (normal saline), which looks like the real thing but is not. It contains no active ingredient.

Kinetics of various potential cardioprotective biomarkers Sub-study – we will be obtaining extra blood samples at the same time of obtaining the original samples to detect markers which could help protecting your heart muscle. We will ask if you would like to participate in the cardioprotection biomarker study. This involves taking some additional blood at three of the main study visits: on the day of surgery; and 2 and 3 days after your surgery. As part of the main study procedures you will have between 2-3 ml of blood collected. If you consent to the cardioprotection biomarker study we will take an extra 1-2 ml of blood at the same time. The total volume of blood taken at these visits is equivalent to 1 teaspoon of blood. There is no additional risk associated to the substudy as we will collect this extra blood at the same time as the sample taken for the main study.

5. What will I have to do?

If you agree to take part in the study and the two sub-studies, there is nothing different you have to do while in hospital. You will need to give the additional bloods samples described above, which will be 6 blood samples in total. Most of these will not involve a separate needle stick. Additionally, if you have private medical insurance, we advise you to inform your insurer as soon as you are able.

6. What are the alternatives for diagnosis or treatment?

Currently there are no alternatives to remote ischaemic preconditioning, other than routine care. There are other drugs under investigation which might protect the heart but they are all in trial stages.

7. What are the possible disadvantages and risks of taking part?

The surgeon or one of the investigators will have explained to you that there are risks involved with any heart surgery. Remote ischaemic preconditioning does not add to the risk of surgery. Glyceryl Trinitrate is a drug that is used frequently in some types of heart surgery and there are few risks associated with its use. It may lower your blood pressure but this eventuality is a risk during heart surgery in general and will be dealt with by the anesthetists. GTN is used

commonly in some kinds of heart surgery, for e.g. coronary artery bypass surgery. It is used less commonly in other forms, for e.g. valve surgery.

Before participating you should consider if this will affect any insurance you have and seek advice if necessary.

8. What are the side effects of any treatment received when taking part?

Given the common use of GTN in this setting, there is a high chance you will receive the drug, even if you do not take part in the study. Thus the risks of the study are likely to be no higher than the risks of the operation as explained to you by the surgeon.

It is possible that if the treatment is given to a pregnant woman it will harm the unborn child. Pregnant women must not therefore take part in this study. Women who are at risk of pregnancy may be asked to have a pregnancy test before taking part to exclude the possibility of pregnancy. Any woman who finds that she has become pregnant while taking part in the study should immediately tell her research doctor.

9. What is the Cardioprotective biomarkers Sub study

This is a sub study that will look into the presence and levels of different markers in your blood at different time pre and post-surgery. We will be using the same bloods samples taken for the main trial for this sub study. No additional blood draws will be taken; we will take only an additional volume of 3-5 mls during the blood draws performed as part of the main study, Some of the samples may be stored for further analysis (up to 2 years before being destroyed)

10. What are the possible benefits of taking part?

We will be able to establish if it is possible to use remote ischaemic preconditioning and Glyceryl Trinitrate in patients undergoing coronary artery bypass graft and valve surgery. The results of the study and the sub study may therefore benefit patients in the future, however, you may not directly benefit in any way from this study.

11. What happens when the research study stops?

Your routine care and the medication that you take are not affected in any way by the treatments in this trial. Once the study stops, you should continue all medicines as advised by your doctor.

12. What if there is a problem?
Any complaint about the way you have been dealt with during the clinical trial or any possible harm you might suffer will be addressed. The detailed information concerning this is given in Part 2 of this information sheet. If you have any concerns or complaints you should contact your study doctor in the first instance.

13. Will my taking part in the study be kept confidential?
Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

14. Contact Details

Your Doctor and Research Fellow

Name Dr Ashraf Hamarneh Tel. Number: 02034479888

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2

15. What if relevant new information becomes available?
Sometimes we get new information about the treatment being studied. If this happens, we will tell you about it and discuss whether you want to or should continue in the study. If you decide not to carry on, your routine care is not affected in any way. If you decide to continue in the study we will ask you to sign an updated consent form.

16. What will happen if I don't want to carry on with the study?
You can withdraw from the study or the sub-study at any time. We request that we be allowed to use any data collected till that point. However, you can ask us to discard all samples and all data collected if you wish.

17. What if there is a problem?

If you think there is a problem while taking part in this trial, please use the following information below to contact the research doctors.

If there is a problem during routine care in the hospital, please inform you nurse or doctors looking after you. They let you know what to do.

Complaints

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Contact details are given on part 1 of this form. If you remain unhappy and wish to complain formally, you can do this via National Health Service or UCL complaints mechanisms. Please ask your research doctor if you would like more information on this.

Every care will be taken in the course of this clinical trial. However in the unlikely event that you are injured by taking part, compensation may be available.

If you suspect that the injury is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation. After discussing with your study doctor, please make the claim in writing to the Professor Derek Yellon who is the Chief Investigator for the clinical trial and is based at The Hatter Cardiovascular Institute, 67 Chenies Mews, University College London, London WC1E 6HX. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of University College London or another party. You should discuss this possibility with your study doctor in the same way as above.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff or about any side effects (adverse events) you may have experienced due to your participation in the clinical trial, the normal National Health Service complaints mechanisms are available to you. Barts Health has a patient advice and liaison service http://www.bartshealth.nhs.uk/pals

They can be contacted via telephone on: **Tel:** 020 3594 2040 or via email at**:** pals@bartshealth.nhs.uk

Please ask your study doctor if you would like more information on this. Details can also be obtained from the Department of Health website: http://www.dh.gov.uk.

18. Will my taking part in this study be kept confidential?

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at your treating hospital and at University College London under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the Sponsor (UCL), who is not involved in the trial. You will be allocated a trial number, which will be used as a code to identify you on all trial forms. Any information about you, which leaves the hospital, will have your name and address removed so that you cannot be recognized.

Your records will be available to people authorised to work on the trial but may also need to be made available to people authorised by the Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

The information collected about you may also be shown to authorised people from the UK Regulatory Authority (the Medicines and Healthcare Products Regulatory Authority); this is to ensure that the study is carried out to the highest possible scientific standards. All will have a duty of confidentiality to you as a research participant.

If you withdraw consent from further study treatment, unless you object, your data and samples will remain on file and will be included in the final study analysis.

In line with the regulations, at the end of the study your data will be securely archived for a minimum of 20 years. Arrangements for confidential destruction will then be made.

19. Will my GP be informed of my involvement?

With your permission, your GP, and other doctors who may be treating you, will be notified that you are taking part in this study.

20. What will happen to any samples I give?

Blood samples will be discarded immediately after the tests have been completed. They will be tested for markers of injury to the heart and kidneys. The additional volume taken for the sub-study will be anonymized and stored at the UCL Hatter Institute for up to 2 years for the cardioprotective biomarkers analyses. These will be destroyed after analysis.

21. Will any genetic tests be done?

No genetic tests will be done

22. What will happen to the results of the research study?

The results of the study will be available after it finishes and will usually be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the patients involved in the trial will be identified in any report or publication.

Should you wish to see the results, or the publication, please ask your study doctor.

23. Who is organising and funding the research?

The research is organized and funded by The Hatter Cardiovascular Institute and is sponsored by University College London. The funding of the Hatter Cardiovascular Institute comes from charities such as the Rosetrees Trust and the National Institute for Health Research. Your doctor will not be paid for including you in this study.

24. Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Westminster Research Ethics Committee.

25. Further information and contact details

You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your study nurse or doctor, who will be able to provide you with up to date information about the drug(s)/procedure(s) involved. If you wish to read the research on which this study is based, please ask your study nurse or doctor. If you require any further information or have any concerns while taking part in the study please contact one of the following people:

Your Doctor and Research Fellow

Name Dr Ashraf Hamarneh Tel. Number: [redacted] *or* [redacted]

Alternatively if you or your relatives have any questions about this study you may wish to contact one of the following organisations that are independent of the hospital at which you are being treated:

If you decide you would like to take part then please read and sign the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.

You can have more time to think this over if you are at all unsure.

Thank you for taking part in this study