ERIC-PPCI

Effect of Remote Ischaemic Conditioning on clinical outcomes in ST segment elevation myocardial infarction patients undergoing Primary Percutaneous Coronary Intervention

Trial Protocol Version 3

Sponsored by UCL

Funded by the British Heart Foundation

Protocol Number: Version 3

Title: **E**ffect of **R**emote **I**schaemic **C**onditioning on clinical outcomes in ST-segment elevation myocardial infarction patients undergoing **P**rimary **P**ercutaneous **C**oronary **I**ntervention (**ERIC-PPCI**): A multicentre randomised controlled clinical trial

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Location: UK Multicentre

ERIC-PPCI is a collaboration with the **Effect of RIC on Clinical Outcomes in STEMI Patients Undergoing pPCI (CONDI2)** trial based in Denmark, Serbia and Spain.

Local sites and investigators: Listed on the ERIC-PPCI website: http://ericppci.lshtm.ac.uk/

Sponsor: University College London

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1. Trial summary

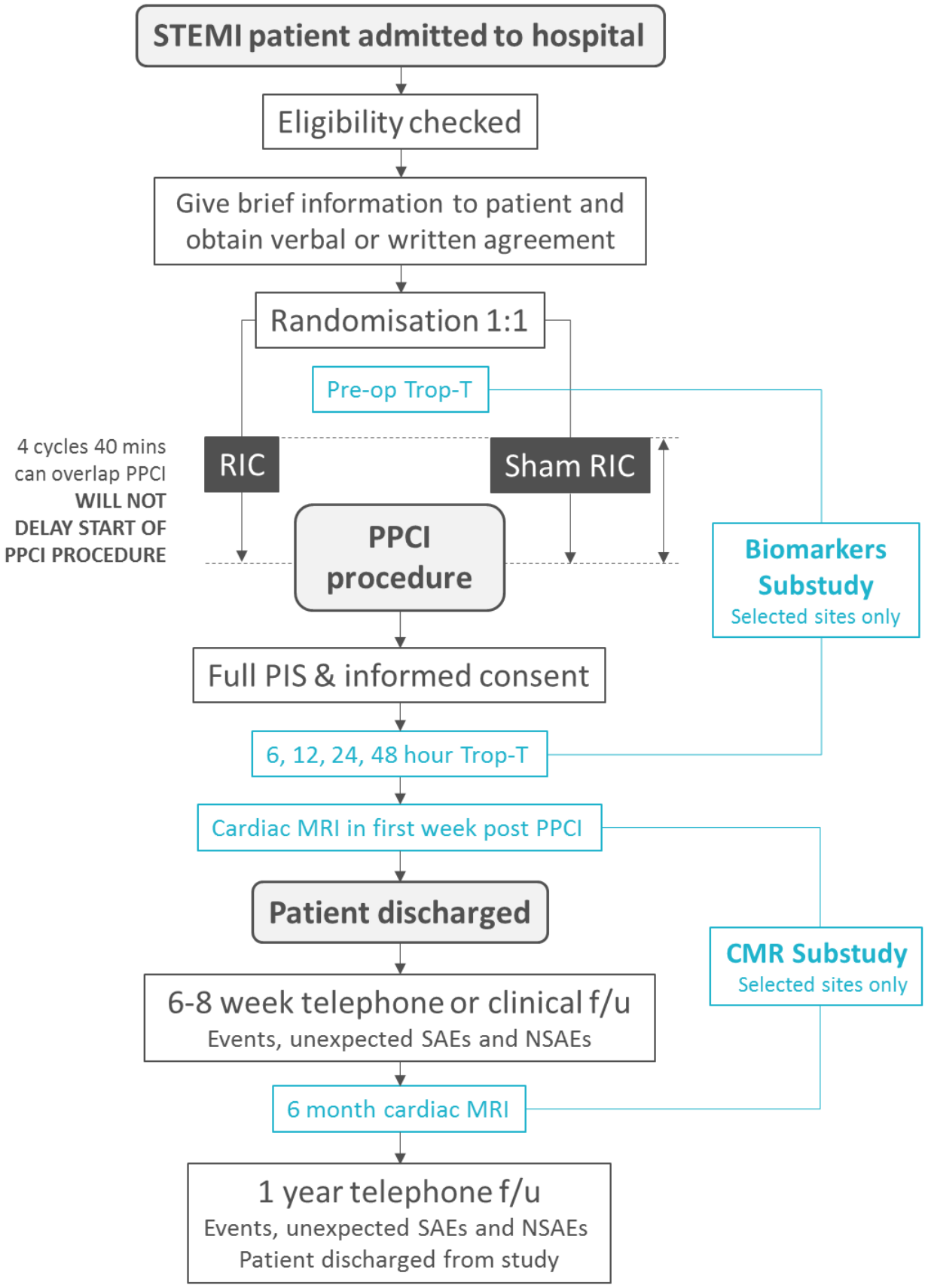
1.1. Protocol summary

Title	**E**ffect of **R**emote **I**schaemic **C**onditioning on clinical outcomes in ST-segment elevation myocardial infarction patients undergoing **P**rimary **P**ercutaneous **C**oronary **I**ntervention (ERIC-PPCI): A multicentre randomised controlled clinical trial.
Sponsor	University College London.
Medical condition or disease under investigation	Acute Myocardial Infarction.
Purpose of clinical trial	To determine whether remote ischaemic conditioning (RIC) improves clinical outcomes in STEMI patients undergoing PPCI.
Trial design	Randomised single blind placebo controlled trial.
Primary objectives	To determine the effect of RIC on cardiac death and hospitalisation for heart failure (HHF) at 12 months in STEMI patients undergoing PPCI.
Secondary objectives	To determine the effects of RIC on: • Rates of cardiac death and HHF at 30 days. • Rates of all-cause death, repeat coronary revascularisation, reinfarction, stroke at 30 days and 12 months. • Quality of life at 6-8 weeks and one year (EQ-5D-5L). • Biomarkers substudy: (400 patients at selected sites) 48 hour area under the curve high-sensitivity troponin T and CK-MB. • CMR Substudy: (250 patients at selected sites) Myocardial infarct size expressed as a percentage of the LV mass by Cardiac MRI at 6 months. • Coronary Physiology Substudy: (180 patients at selected sites) Index of microcirculatory resistance (IMR) during and on completion of PPCI. • Thrombosis Substudy: (all patients recruited at Lister Hospital) Global Thrombosis Test (GTT) and tests of thrombogenesis and fibrinolysis using micro-titre based assays on fresh frozen plasma (FFP). • Myosin C Substudy: (24 patients at Barts Heart Centre) 48 hour area under the curve cMyBP-C.
Sample size	**ERIC-PPCI trial** (UK) 2000 patients undergoing PPCI following STEMI.

	The trial is a collaboration with the CONDI2 trial (Denmark, Serbia, Spain) which will recruit 2300 patients (NCT01857414). In total the sample size is 4300 patients.
Inclusion criteria	1. Onset of STEMI symptoms within 12 hours, lasting for more than 30 minutes 2. Age >18 years 3. Suspected STEMI (ST-elevation at the J-point in two contiguous leads with the cut-off points: ≥0.2 millivolt (mV) in men or ≥0.15 mV in women in leads V2-V3 and/or ≥0.1 mV in other leads.) 4. Eligibility for coronary angiography with follow on PPCI if indicated
Exclusion criteria	1. Previous coronary artery bypass graft surgery 2. Myocardial infarction (MI) within the previous 30 days 3. Treatment with thrombolysis within the previous 30 days 4. Left bundle branch block 5. Patients treated with therapeutic hypothermia 6. Conditions precluding use of RIC (paresis of upper limb, use of an a-v shunt) 7. Life expectancy of less than 1 year due to non-cardiac pathology 8. Previous entry into the ERIC-PPCI trial
Trial treatment	The intervention will be applied at a single time point on arrival at the PCI centre, starting prior to the PPCI procedure. The trial treatment may overlap with the start of the PPCI procedure and will not delay PPCI. Active treatment (RIC) will consist of four 5 minute inflations of an automated autoRIC™ cuff or manual blood pressure cuff on the upper arm to 200mmHg. For patients presenting with a systolic blood pressure (SBP)≥175mmHg, a manual blood pressure cuff will be used in place of the autoRIC™ cuff. This will be inflated to 25 mmHg above systolic blood pressure. The inflations will be separated by 5 minute periods when the blood pressure cuff will be deflated. Control treatment (sham RIC) will consist of placing an identical looking autoRIC™ cuff on the upper arm which is designed to deliver four 5 minute simulated inflations, or will be delivered using a manual blood pressure cuff. The simulated inflations will be separated by 5 minute periods when the blood pressure cuff will remain uninflated.

A glossary of terms and abbreviations used in this protocol is included as **Appendix 2**.

1.2. Trial flowchart



2. Introduction

2.1. Background

Ischaemic heart disease, the leading cause of death in the UK, accounts for 90,000 deaths per year and costs the UK economy nearly £9 billion per annum. Each year 150,000 patients have an acute myocardial infarction (BHF Stats 2010). In patients presenting with an ST-elevation myocardial infarction (STEMI), early myocardial reperfusion using primary percutaneous coronary intervention (PPCI) is the most effective therapeutic intervention for limiting myocardial infarct (MI) size, a major determinant of prognosis post-PPCI. However, despite PPCI, the morbidity and mortality of STEMI patients remain significant, paving the way for novel therapeutic strategies for protecting the heart against acute ischaemia-reperfusion injury (IRI). In this respect remote ischaemic conditioning (RIC) represents a non-invasive and low cost therapeutic strategy for further reducing MI size, preventing the onset of heart failure, and improving clinical outcomes in PPCI patients. For systematic reviews on RIC please see the following references[1, 2].

2.2. Scientific rationale

RIC describes the phenomenon in which the application of multiple cycles of brief non-lethal ischaemia and reperfusion to an organ or tissue remote from the heart protects the myocardium from a lethal sustained episode of acute IRI[1, 2]. The mechanisms underlying RIC are unclear but have been attributed to a neuro-hormonal pathway linking the preconditioned organ or tissue to the heart [2-4]. In 2002, Kharbanda et al[5] first demonstrated that the RIC stimulus could be induced non-invasively in human volunteers by simply inflating and deflating a blood pressure cuff placed on the upper arm. We and others have shown that this RIC stimulus is beneficial in reducing perioperative myocardial injury in the settings of cardiac bypass and abdominal aortic aneurysm surgery[6-9], and reducing periprocedural myocardial and renal injury in the setting of elective PCI[10, 11]. However, several recent studies have reported neutral findings with RIC in these clinical settings[12-15]. The reasons for this are unclear but have been attributed to differences in study design, patient selection and concomitant medication (which are known to interfere with RIC cardioprotection)[16]. Preliminary data suggest that long term outcomes may be improved in RIC treated CABG patients[17], although this needs to be confirmed in prospective large randomised controlled clinical trials such as ERICCA[18] and RIPHeart,[19] which have been prospectively designed and powered to investigate the effect of RIC on long term clinical outcomes in patients undergoing a coronary artery bypass graft operation.

RIC has now been investigated in four small proof-of-concept clinical studies in STEMI patients undergoing PPCI including one by this research group[20-24]. The first study was published by Botker et al (the research collaborator in Denmark)[20, 21] and reported that RIC (four 5 minute upper arm cuff inflations and deflations) administered in the ambulance by paramedics on route to the PPCI centre, significantly increased the mean salvage index from 0·57 in control to 0.69 with RIC at 30 days (as measured by myocardial SPECT). In a post-hoc subgroup analysis of patients presenting with a left anterior descending STEMI, myocardial salvage was increased further, and there was a significant reduction in final MI size and improvement in LV ejection fraction at 30 days[20, 21]. Interestingly, 6 year follow up of this patient cohort revealed less all-cause death in those patients given RIC at the time of their PPCI, although this study has not been prospectively designed to investigate long term endpoints [25]. In another study of 96 patients, Rentoukas et al[22] demonstrated that RIC (three 4 minute upper arm cuff inflations and deflations) administered at the PPCI centre improved ST-segment resolution and reduced MI size when compared to control. Crimi et al [23] have found that RIC administered at the onset of myocardial reperfusion was beneficial in STEMI patients undergoing PPCI.

2.3. Research collaboration with Denmark

The ERIC-PPCI trial will be conducted in collaboration with Prof Hans Erik Bøtker (Aarhus University, Denmark), who is CI of the Effect of RIC on Clinical Outcomes in STEMI Patients Undergoing pPCI (CONDI2) trial (NCT01857414).

The CONDI 1 trial (CI Hans Bøtker) was the first to successfully demonstrate a beneficial effect of RIC in 142 STEMI patients undergoing PPCI in terms of increased myocardial salvage and smaller MI size [20], effects which were present 6 months later in terms of improved LV systolic function [26]. A €3.0 million research grant has been awarded by the Danish Research Council to investigate the effect of RIC on 12 month clinical outcomes in STEMI patients undergoing PPCI.

The results of ERIC-PPCI and CONDI 2 will be combined in order to maximise power to assess the impact of RIC on clinical endpoints.

The ERIC-PPCI and CONDI 2 trials will each have a separate DSMC and TSC although there will be an opportunity for the these committees to contact each other if required.

A combined and independent Endpoint Validation Committee will review death, HHF, stroke and MI from both trials.

3. Hypothesis

Remote ischaemic conditioning improves long term clinical outcomes (cardiac death and hospitalisation for heart failure) at 12 months in STEMI patients undergoing PPCI.

4. Endpoints

4.1. Primary endpoint

To investigate whether RIC reduces the combined primary endpoint rate of cardiac death and hospitalisation for heart failure (HHF) at 12 months in STEMI patients undergoing PPCI.

4.1.1. Definition for cardiac death

All deaths where there is no clinical or post mortem evidence of a non-cardiac aetiology.

4.1.2. Definition for hospitalisation for heart failure

This will include both heart failure during the index hospitalisation and re-hospitalisation for heart failure. Hospitalisation will be defined as a treatment occurring in hospital. Heart failure will be judged to be present on symptoms (at least one of the following: new or worsening dyspnea, orthopnea, paroxysmal nocturnal dyspnea or increasing fatigue/worsening exercise tolerance) and signs (one of the following: new pulmonary oedema by chest X-ray in the absence of a non-cardiac cause, crepitations believed to be due to pulmonary oedema, and use of loop diuretics to treat presumed pulmonary congestion)[41]. Probable HHF will be recorded if there are features suggestive of a diagnosis of heart failure but insufficient evidence to be considered definite HHF. Both definite and probable HHF will be included in the primary endpoint.

4.2. Secondary endpoints

To investigate whether RIC can affect the following secondary endpoints below:

1. Rates of cardiac death and HHF at 30 days. This data will be collected at the 6-8 weeks post-randomisation follow-up.
2. Rates of all-cause death, repeat coronary revascularisation, reinfarction, stroke at 30 days and 12 months. This data will be collected at the 6-8 weeks and one year post-randomisation).

3. Quality of life at 6-8 weeks and 12 months (EuroQol EQ-5D-5L) (for an example EQ-5D-5L see **Appendix 3**)

Biomarkers substudy (400 patients at selected sites)

4. MI size on 48 hour area-under-the-curve (AUC) hsTrop T and CK-MB

MRI substudy (250 patients at selected sites)

5. MI size on 6 months cardiac MRI (new late gadolinium enhancement expressed as a percentage of the LV mass).
6. Microvascular obstruction on cardiac MRI (hypodense area on late gadolinium enhancement).
7. Myocardial salvage index (AAR [T2 weighted imaging or angiography jeopardy score] subtract final MI size).
8. LV remodelling on 6 month cardiac MRI scan:
    - index LV end-diastolic and end-systolic volumes
    - LV ejection fraction
    - LV mass and wall thickness

Coronary Physiology substudy (180 patients at selected sites)

9. Index of microcirculatory resistance (IMR) during and on completion of PPCI

Thrombosis substudy (all patients recruited at Lister Hospital)

10. Thrombotic status pre and post-PPCI
11. Change in thrombotic status pre and post intervention
12. Differences in ECG ST-resolution and clinical outcomes

Myosin C substudy (24 patients at Barts Heart Centre)

14. MI size on 48 hour area-under-the-curve (AUC) Myosin C

4.2.1. Definition of stroke

Stroke is defined as a focal, central neurological deficit lasting >72 hours which results in irreversible brain damage or body impairment. Probable stroke will be recorded if there are features suggestive of this but insufficient evidence to classify as definite stroke.

Both definite and probable stroke will be included in the secondary endpoint.

4.2.2. Definition of reinfarction

Reinfarction is defined as an acute MI that occurs within 28 days of an incident or recurrent MI. The ECG diagnosis of suspected reinfarction following the initial MI may be confounded by the initial evolutionary ECG changes. Reinfarction should be considered when ST elevation >0.1 mV recurs, or new pathognomonic Q waves appear, in at least two contiguous leads, particularly when associated with ischaemic symptoms for 20 min or longer. Re-elevation of the ST-segment can, however, also be seen in threatened myocardial rupture and should lead to additional diagnostic workup. ST depression or LBBB alone are nonspecific findings and should not be used to diagnose reinfarction.

In patients in whom reinfarction is suspected from clinical signs or symptoms following the initial MI, an immediate measurement of cTn is recommended. A second sample should be obtained 3–6 h later. If the cTn concentration is elevated, but stable or decreasing at the time of suspected reinfarction, the diagnosis of reinfarction requires a 20% or greater increase of the cTn value in the second sample. If the initial cTn concentration is normal, the criteria for new acute MI apply.

4.2.3. Definition of coronary revascularisation

Revascularisation is defined as coronary revascularisation by PCI or CABG following index PPCI, excluding staged PPCI.

4.3. Endpoint Validation

The primary combined endpoint, stroke, MI, reinfarction and revascularisation will be validated by an independent combined event validation committee (EVC), which will validate death, HHF, MI and strokes occurring in both ERIC-PPCI and CONDI 2. The EVC will be blinded to the randomised treatment allocation.

5. Power calculations and sample size determination

5.1. Primary combined clinical endpoint

The primary combined endpoint will be cardiac death and HHF at 12 months. These endpoints have been selected as they are the most relevant clinical endpoints that are likely to be affected by RIC, an intervention which protects cardiomyocytes against acute IRI. According to the UK NIAP 2008 database, cardiac mortality at 12 months ranged from 5.8%-16.7% depending on the call to balloon time, with the overall 12 month death rate of 8.7% for all PPCI patients. In a recent Danish clinical study post-PPCI, the one year mortality was 9.4% and the cumulative risk of readmission with heart failure was 8% [30]. In another, non-UK based, clinical study, the incidence of HHF was 12.7% at 12 months post-PPCI [31]. We have based our power calculations on these published studies, accounting for the marked improvements in clinical outcomes in the contemporary era by using much more conservative event rates.

As a conservative estimate we will use a combined cardiac death and HHF event rate of 11.0% at 12 months for all-comer STEMI patients. In the combined ERIC-PPCI / CONDI 2 trial we estimate the effect size to be a 25% relative reduction in the event rate. The rationale for this is based on proof-of-concept clinical studies in which RIC, and related therapeutic interventions such as ischaemic postconditioning, have reported 40-50% reductions in MI size[32]. To demonstrate a 25% reduction in the primary composite endpoint in the RIC-treated group (from 11.0% to 8.25%), with 80% power and at the 5% significance level, will require 1805 patients per treatment arm which equates to 3610 patients in total. Therefore, we will need to recruit 4300 STEMI patients (allowing for a 15% drop out rate at 12 months) between UK and Denmark (2000 STEMI patients in the UK and 2300 STEMI patients in Denmark). Therefore, in the ERIC-PPCI trial we intend to recruit 2000 STEMI patients in the UK through 30 PPCI sites.

If the event rate in the control arm is higher than the estimated 11% or the losses less than 15% then the power will increase. The ERIC-PPCI UK arm of the trial of 2000 patients alone would provide 80% power to detect a reduction of approximately a third from 11.0% to 7.3% allowing for 5% losses to follow up.

5.2. Biomarker substudy to assess the effect of RIC on MI size (48 hr AUC)

A major secondary endpoint of the ERIC-PPCI study will be MI size as measured by 48 hr AUC high-sensitive Troponin T and CK-MB. To date the effect of RIC on this specific endpoint has not been investigated. Using 72 hr AUC Trop I, Thibault et al [32] demonstrated that ischaemic postconditioning reduced MI size from 24.6 (SD 20.6) $x10^4$ to 13.0 (SD 7.0) $x10^4$ IU/L. This equates to a relative reduction in MI size of 47%. In order to demonstrate a more conservative 25% relative reduction in MI size with RIC in PPCI patients from 24.6 (SD 20.6) $x10^4$ using 48 hr AUC hsTrop T with 80% power and at the 5% significance level, will require 177 patients in each treatment group or 354 patients in total. Allowing for 10% dropout rate would require 400 patients in total.

In view of the clear positive skewness of the Troponin AUC it is anticipated that this outcome will be analysed on the log scale. This will lead to estimation of the relative reduction in the MI size, which is expected to be a more precise estimate of any treatment effect. Therefore, the planned sample size of 400 patients is likely to provide greater than 80% power and allow for larger than a 10% loss in outcomes due to incomplete Troponin AUCs.

5.3. CMR substudy to assess the effect of RIC on MI size at 6 months

The CMR substudy primary endpoint is MI size (mass of late gadolinium enhancement) expressed as percentage of LV mass at 6 months. No clinical studies have previously used cardiac MRI to assess the effect of RIC on MI size. Using myocardial SPECT, Botker et al [20] were unable to demonstrate a significant reduction in MI size at 30 days in all STEMI patients. Lonborg et al [33] used CMR to demonstrate that ischaemic postconditioning reduced MI size at 3 months post-PPCI from 17±8% to 14±7% (mass of infarct expressed as a % of the LV mass). This equates to a relative reduction in MI size of 17.6% (equivalent to an absolute reduction of 3%). Based on this data, to demonstrate a similar relative reduction in MI size with RIC in PPCI patients from 17% (SD 8%) in the 6 month CMR scan, with 80% power and at the 5% significance level, will require approximately 112 patients in each treatment group or 224 patients in total. To allow for a 10% dropout rate we plan to recruit 250 patients in total.

6. Selection of patients

6.1. Inclusion criteria

1. Onset of STEMI symptoms within 12 hours, lasting for more than 30 minutes
2. Patients older than 18 years
3. Suspected STEMI (ST-elevation at the J-point in two contiguous leads with the cut-off points: ≥0.2 millivolt (mV) in men or ≥0.15 mV in women in leads V2-V3 and/or ≥0.1 mV in other leads)
4. Eligibility for coronary angiography with follow on PPCI if indicated

6.2. Exclusion criteria

1. Previous coronary artery bypass graft surgery
2. Myocardial infarction (MI) within the previous 30 days
3. Treatment with thrombolysis within the previous 30 days
4. Left bundle branch block (LBBB)
5. Patients treated with therapeutic hypothermia
6. Conditions precluding use of RIC (paresis of upper limb, use of an a-v shunt)
7. Life expectancy of less than 1 year due to non-cardiac pathology
8. Previous entry into the ERIC-PPCI trial

For centres recruiting into CMR substudy only: Please refer to section 13 as additional exclusion criteria apply to substudy patients.

6.3. Patients entered into observational research

Patients may be entered into registries or observational studies while also participating in ERIC-PPCI.

7. Ethical considerations

7.1. Consent

As patients admitted with a STEMI will need to receive urgent treatment, randomisation and the trial intervention will need to be started as early as possible to ensure that there is no delay to the PPCI procedure. The physical, mental and emotional state of patients may also be affected due to their MI and the use of analgesic drugs in pain management, which could impair their decision making capacity. Therefore, the consent process in this situation requires careful consideration bearing in mind applicable regulatory requirements, adherence to ICH-GCP and the requirements in the Declaration of Helskini.

7.1.1. Patient agreement

The patient will be approached at the time that their STEMI is confirmed and information about the trial should be provided to the patient to their level of capacity. A patient leaflet is provided for this purpose. The patient leaflet may be signed by the patient if they agree to participate, however verbal assent is the only requirement. If the patient cannot give verbal assent they must not be entered into the trial.

Once agreement is obtained the patient may be entered into the trial. Agreement to take part in the trial should be recorded in the patient notes. A signed patient leaflet is not mandatory and will not be considered as equivalent to informed consent.

7.1.2. Personal Consultee

When during the PPCI procedure a personal consultee is present:

A friend or relative's advice should be taken on whether they think the patient would wish to be part of the trial, particularly if the patient had expressed any prior wishes or advanced decisions. A consultee declaration form should be completed. Once the patient has recovered they should still be approached to give informed consent regardless of whether a consultee declaration form has been completed.

When a patient has agreed but does not regain capacity to give informed consent:

After the PPCI procedure a friend or relative's advice should be taken on whether they think the patient would wish to be part of the trial, particularly if the patient had expressed any prior wishes or advanced decisions. A consultee declaration form should be completed.

7.1.3. Informed consent

As soon as the patient has recovered after the procedure the patient should be given sufficient time to consider the trial and ask questions, following which informed consent will be taken. If the patient elects to withdraw from the trial at this point then we will seek consent to use the data and samples already acquired.

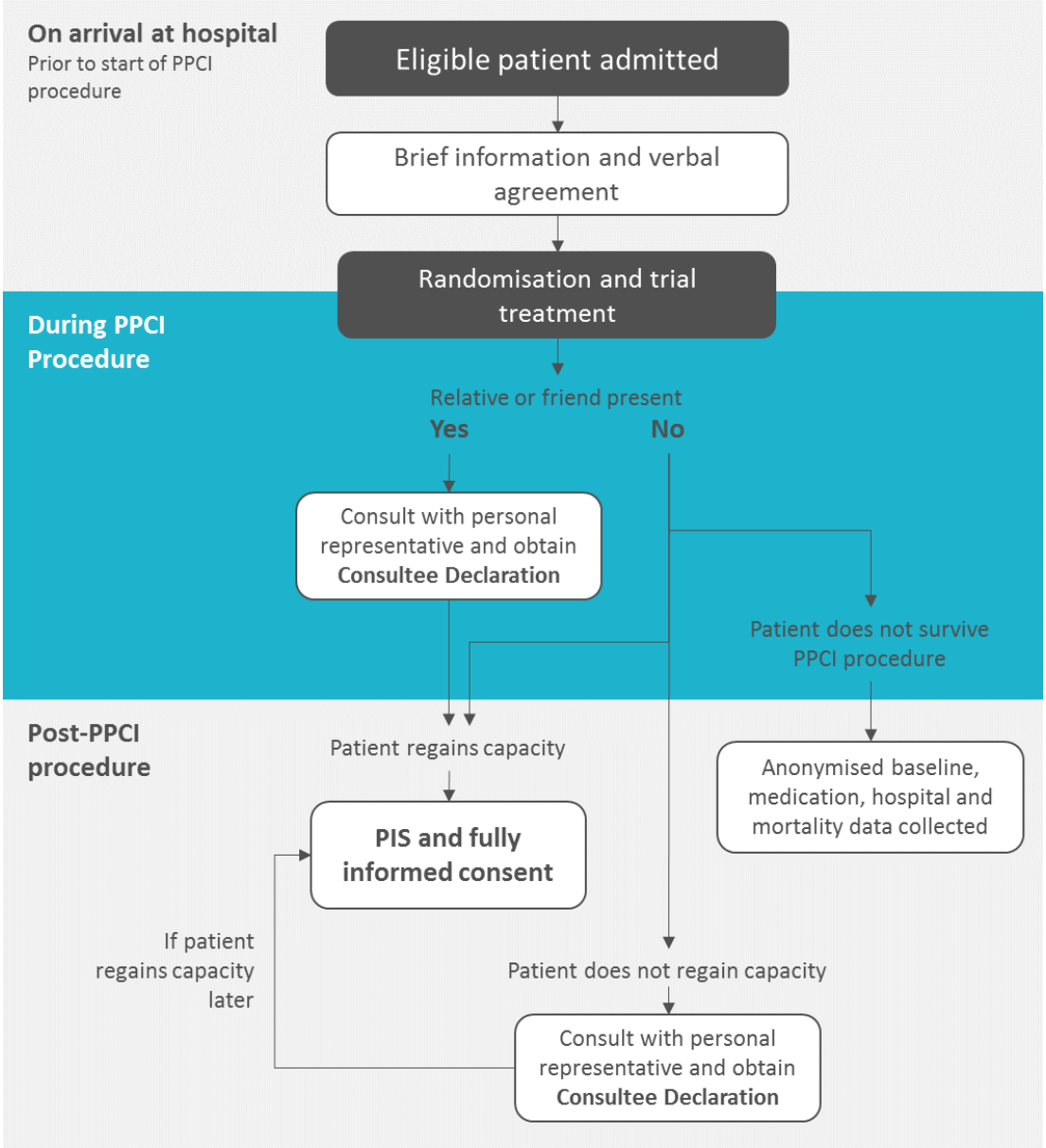
7.1.4. Withdrawal

A patient may decide to withdraw from the trial at any time without prejudice to their future care.

7.1.5. Patients not surviving the PPCI procedure

Written consent will not be available for those patients that die during the PPCI procedure. Permission has been granted by the Confidentiality Advisory Group (CAG) to allow us to use non-identifiable information gathered up to that point in the trial. The CAG reference number is 15/CAG/0150.

7.1.6. Consent flowchart



7.2. Declaration of Helsinki and UCL Good Clinical Practice

The trial will conform to the spirit and the letter of the declaration of Helsinki, and in accordance with the UCL Good Clinical Practice Guidelines.

7.3. Ethical committee review

NRES Committee London-Harrow have reviewed and approved the trial. The REC number is 15/LO/0217. Copies of the letters of approval will be filed in the trial site files at each centre.

8. Randomisation

8.1. Randomisation procedure

Patients will be randomised to either RIC or sham RIC by a designated research investigator, this investigator will therefore be unblinded to treatment allocation. Randomisation will be coordinated centrally by the LSHTM CTU via a secure website www.sealedenvelope.com and will be stratified by centre using random permuted blocks.

8.2. Access to randomisation site

Access to the randomisation website will be strictly controlled at each site and limited to unblinded research investigators delegated by the PI to be responsible for performing either the RIC or sham RIC protocol.

Patients will be randomised to receive either **RIC** or **sham RIC**.

9. Blinding

9.1. Unblinded trial staff

At each site specific staff will be delegated by the PI to perform the randomisation and intervention procedures. These staff will be the only people in each centre aware of the treatment allocation for the patient and will not be involved with data collection other than any relating to the randomisation and intervention procedures.

At the LSHTM clinical trials unit the unblinded trial statistician and the data managers will have access to allocation data.

9.2. Blinded trial staff

The patients and the research doctor/nurse collecting the data and following up the patient will be blinded to the treatment allocation. Outcome assessments will be blinded. The event validation committee will be blinded to treatment allocation.

In the CMR substudy the research fellow analysing the CMR scan will be blinded to the treatment allocation.

9.3. Emergency unblinding

The benign and short term nature of the intervention makes the need for emergency unblinding unlikely, if required patients will be able to be unblinded through the randomisation website.

10. Trial treatment

Patients will be randomised on arrival at the hospital and the unblinded research investigator will then deliver either the RIC or sham RIC protocol.

Automated CellAegis autoRIC™ cuff devices www.cellaegisdevices.com will be supplied to deliver the RIC and sham RIC protocols. A manual cuff (sphygmomanometer) will also be supplied to each site, as backup for the autoRIC™ device and to deliver the trial treatment for patients with a systolic blood pressure (SBP) ≥175mmHg.

AutoRIC™ devices are used in conjunction with single use disposable inner cuffs. An autoRIC™ device, an autoRIC™ sham device and disposable cuffs will be provided to the participating sites.

The advantage of the autoRIC™ device is that once it has been placed on the upper arm, the preprogrammed standard RIC or sham RIC protocol is simply delivered by pressing a single start button.

10.1. Remote ischaemic conditioning (RIC)

An automated autoRIC™ cuff, or manual cuff, will be placed on the upper arm and inflated to 200mmHg for 5 minutes and then deflated for 5 minutes, a cycle which will be undertaken 4 times in total. For patients presenting with SBP ≥175mmHg, a manual blood pressure cuff will be used and inflated to 25 mmHg above systolic blood pressure.

10.2. Sham RIC

An autoRIC™ cuff visually identical to that used in the RIC protocol, or manual cuff, will be placed on the upper arm and a simulated RIC protocol applied. Inflation will be simulated and held for 5 minutes, deflation will then be simulated and held for five minutes, a cycle which will be undertaken 4 times in total.

The sham device's components and external appearance are identical to that of the autoRIC™. However, as compared to the autoRIC™, the sham device control unit's pump is disconnected such that the control unit cannot inflate the applicator cuff. The sham device provides the same sound and vibration as that of the pump inflating and the same LED indicators on the control unit. The operation of the autoRIC™ control unit with respect to the (simulated) RIC procedure initiation, cycle indication, and termination are identical in the sham device, with the exception that the applicator cuff does not inflate.

10.3. Initiation of treatment

The trial treatment should be started with sufficient time to ensure that as many cycles of ischaemia and reperfusion as possible are completed before the onset of reperfusion.

10.4. Duration of treatment

Although the RIC / sham RIC protocol lasts 40 minutes in total, the cuff should be removed after the 4th cycle of inflation and the last 5 min of reperfusion undertaken with the cuff removed. This means that after 35 min the intervention is completed. In the cases where the door to PPCI time is less than 35 min, the RIC / sham RIC protocol may overlap with the beginning of the PPCI procedure when the coronary angiogram is being performed.

Under no circumstances should the RIC protocol delay the onset of the PPCI procedure.

11. Data collection and follow up

11.1. Trial procedures table

	pre-PPCI	PPCI	Post-PPCI in hospital					After discharge		
		0 hrs	6 hrs	12 hrs	24 hrs	48 hrs	2-7 days	6-8 weeks	6 months	12 months
Clinical assessments										
Review of eligibility criteria	X									
Patient agreement	X									
History and examination	X									
PIS & Informed consent			**Prior to patient discharge**							
Trial intervention										
Randomisation	X									
RIC / Sham RIC*	X (May overlap with PPCI)									
Clinical outcomes										
Death		X	X					X		X
HHF			X					X		X
MI		X	X					X		X
Revascularisation			X					X		X
Stroke		X	X					X		X
Quality of life										
EQ-5D-5L								X		X
Safety reporting										
SAE / NSAEs		X	X					X		X
Substudies (at selected sites only)										
Biomarker substudy: Trop-T and CK-MB		X	X	X	X	X				
CMR substudy: Cardiac MRI							X		X	
Coronary Physiology substudy: IMR		X								
Thrombosis substudy		X			X	2-3 days post-randomisation		X		
Myosin-C substudy		X	X	X	X	X				

* The RIC/Sham RIC may overlap with the PPCI procedure and should not delay the start of PPCI

11.2. Data collection

All patients will have a full medical history taken and various clinical examinations as part of usual care. The following are to be recorded on the trial CRF:

- Weight and height (BMI will be calculated automatically when entered on the eCRF)
- Blood Pressure
- Gender
- Ethnicity
- Date of birth
- Medical history:
  - Known Diabetes Mellitus
  - Hypercholesterolaemia
  - Hypertension
  - Previous myocardial infarction
  - Previous PCI
  - Previous CABG
  - Previous stroke
  - Atrial fibrillation
  - Peripheral arterial disease
  - Smoking history
  - Family history of IHD
  - History of renal disease
  - Other cardiac disease
- NYHA class
- Ejection fraction post procedure
- Analgesia use
- Medication at admission and discharge:
  - Antiplatelets
  - β-blockers
  - ACE inhibitors
  - Angiotension receptor blockers
  - Calcium channel blockers
  - Digoxin
  - Anti-diabetic drugs
  - Lipid lowering drugs
  - Anticoagulants
  - Diuretics
  - Antianginal drugs
- ECG at admission and prior to discharge
- Call to balloon time, door to balloon time and symptoms to balloon time
- Angiographic data (TIMI flow pre and post-PPCI)
- Use of thrombectomy
- Details of the PPCI procedure
- Procedural drugs
- NHS number. Mortality data will be tracked up to 10 years after randomisation.
- GCS on admission
- Killip Class
- Out of hospital cardiac arrest

- Ventilated pre procedure
- Arterial gas taken
- Creatinine

11.3. Trial procedures

On admission

- Review of eligibility criteria
- Provide information to patient's level of capacity
- Patient agreement (please refer to section 7.1)
- Randomisation
- Randomly allocated trial intervention – RIC or sham RIC
- Baseline Troponin-T/CK-MB
- Thrombosis substudy only: baseline point-of-care Global Thrombosis Test (GTT)
- Myosin C substudy only: baseline Myosin C

Post-PPCI

- Full Patient Information Sheet
- Informed consent
- Biomarker substudy only: 6, 12, 24 and 48 hour Troponin-T/CK-MB
- CMR substudy only: 2-7 day cardiac MRI
- Coronary Physiology substudy: IMR before and after stent placement
- Thrombosis substudy only: 24 and 48-72 hour point-of-care GTT
- Myosin C substudy only: 6, 12, 24 and 48 hour Myosin C
- Events
  - Death
  - Heart failure during the index hospitalisation
  - MI
  - Stroke
- Unexpected SAEs and NSAEs
- Bleeding
- Cardiac medication

6-8 weeks post-randomisation (telephone or outpatient follow-up)

- Events
  - Death
  - Hospitalisation for heart failure
  - MI
  - Stroke
  - Revascularisation
- Quality of life (EQ-5D-5L)
- Unexpected SAEs and NSAEs
- Device implantation
- Ejection fraction (if known)
- Cardiac medication
- Thrombosis substudy only: 6-8 week point-of-care GTT

6 months post-randomisation

- CMR Substudy only: 6 month cardiac MRI (outpatient appointment)

12 months post-randomisation (telephone follow up)

- Events
  - Death
  - Hospitalisation for heart failure
  - MI
  - Stroke
  - Revascularisation
- Quality of life (EQ-5D-5L)
- Unexpected SAEs and NSAEs
- Device implantation

11.4. Compliance and loss to follow up

Problems with compliance are expected to be rare given that the intervention is non-invasive in nature and is administered at a single time point.

Patients are free to withdraw from the trial at any time without prejudice to their future care. Loss to follow up is expected to be rare as the trial is designed to be minimally disruptive to participants. Data collected up to the point of withdrawal will be used unless the patient specifically requests that it is not.

The patients will be followed up at 6-8 weeks and 12 months after PPCI, in order to determine endpoints contributing to the primary endpoint (cardiac death and hospitalisation for heart failure).

These follow ups will either be planned to coincide with existing clinical appointments or will otherwise be conducted by telephone. A non-compliance and dropout rate of 15% has been accounted for in the sample size.

12. Biomarker substudy

MI size as measured as the 48 hour AUC serum level of high-sensitive Troponin-T (hsTropT) and CK-MB will be analysed in 400 patients. After the data has been acquired for the MI size in 400 patients a prespecified interim analysis will be performed.

12.1. Procedure

From each patient, a single blood sample will be taken at each of the 5 timepoints (0, 6, 12, 24 and 48 hours following PPCI procedure). Each blood sample will be analysed at the local hospital.

12.2. Analysis methods

Quantitative serum hsTropT measurement will be performed using a one step immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche, Switzerland). The reference range will be ≤14 ng/L (14 ng/L is the 99th centile of reference population with cardiovascular risk of <10%).

Quantitative serum CK-MB measurement will be performed using a standard immunoassay method.

13. CMR substudy

The CMR substudy will recruit 250 PPCI patients through a selection of PPCI centres with facilities for performing CMR scans in PPCI patients. All sites performing CMR for the ERIC-PPCI study will have a Siemens or Philips 1.5 T scanner and will use a standardised CMR protocol which is included in this protocol as appendix 1.

Training in the CMR protocol will be provided to each recruiting site by the ERIC-PPCI research fellow. Each patient will receive two CMR scans, the first performed within the week following the PPCI procedure and the second at

6 months. All CMR scans will be analysed at a central CMR core lab which will be staffed by an independently funded senior CMR clinical fellow.

The ERIC-PPCI CMR substudy will also put the research infrastructure in place for future PPCI/CMR clinical studies in the UK.

13.1. CMR substudy endpoints

The primary endpoint of the CMR substudy will be MI size on the 6 month CMR scan (measured in mass of late gadolinium enhancement and expressed as a percentage of the LV mass).

Several other CMR parameters will be collected as follows:

13.1.1. The acute post-PPCI CMR scan

1. Left ventricle (LV) ejection fraction and indexed LV end systolic and diastolic volumes and mass using short axis SSFP cine imaging.
2. MI size measured by the mass of late gadolinium enhancement (20 min after administration of contrast) of cardiac MRI scan expressed as a percentage of LV mass.
3. Area at risk (AAR) measured as the increase in T2 values using a Siemens T2 mapping sequence, which has been validated against conventional measures of area at risk [35-37]. The AAR will also be estimated using the modified BARI and APPROACH angiography scores [38].
4. Myocardial salvage index = AAR subtract MI size/AAR. The myocardial salvage index (using T2-weighted CMR and late gadolinium enhancement) has been demonstrated to predict prognosis post-PPCI [39].
5. The incidence and extent of microvascular obstruction (hypo-enhancement on late gadolinium enhancement 20 min after administration of contrast).
6. The incidence and extent of intramyocardial haemorrhage (hypo-enhancement on Siemens T2* mapping sequence)[40].

13.1.2. The follow up CMR scan 6 months post-PPCI

1. LV ejection fraction and indexed LV end systolic and diastolic volumes and mass.
2. MI size measured by the mass of late gadolinium enhancement.

13.2. Exclusion criteria for CMR substudy

Known contraindication to cardiac magnetic resonance imaging (MRI) such as:

1. Significant claustrophobia
2. Severe allergy to gadolinium chelate contrast
3. Severe renal insufficiency (defined as estimated glomerular filtration rate [eGFR] <30 mL/min/1.73 m2)
4. Presence of MRI contraindicated implanted devices (e.g. pacemaker, implanted cardiac defibrillator, cardiac resynchronisation therapy device, cochlear implant)
5. Embedded metal objects (e.g., shrapnel)
6. Any other contraindication for cardiac MRI

Patients may still enroll in the main trial without enrolling in the CMR substudy.

14. Safety Reporting

14.1. Definition

Unexpected events that have not been defined as endpoints (section 4), expected complications of the RIC stimulus or expected complications of usual clinical care (section 14.3) should be reported as either an SAE or NSAE, depending on their severity. Safety reporting for each patient should commence from time of randomisation to completion of follow up at one year after the PPCI procedure.

14.2. Expected adverse events (recognised to be caused by the RIC stimulus)

The benign nature of the RIC stimulus excludes there being any expected serious adverse events. The following are expected non-serious events in response to the RIC stimulus and will be recorded on the Case Report Form. They do not need to be reported to the Clinical Trials Unit.

1. Skin petechiae caused by cuff inflation

14.3. Expected serious adverse events related to usual clinical care

These events are recognised complications of PPCI. They will be recorded on the Case Report Form but do not need to be reported separately on an SAE form:

1. Death
2. Acute renal failure which may require haemodialysis, peritoneal dialysis, or haemofiltration
3. Ventricular tachycardia of fibrillation requiring direct-current (DC) cardioversion
4. Significant heart block requiring temporary or permanent cardiac pacing
5. Tamponade requiring urgent surgical intervention
6. Cardiogenic shock requiring intra-aortic balloon pump or other assist devices

The following events are recognised complications of routine clinical care and for the purposes of this trial will not be designated as SAEs. They do not need to be reported:

1. Atrial fibrillation
2. Acute mitral valve cordal rupture or ventricular septal rupture requiring surgical intervention
3. Persistent complete heart block requiring permanent pacemaker implantation
4. Aspiration pneumonia following VF arrest
5. Rib fracture following chest compression

14.4. Unexpected Serious Adverse Events

Any untoward medical occurrence/effect that:

1. Results in death
2. Is life-threatening*
3. Requires hospitalisation or prolongation of existing inpatient's hospitalisation
4. Results in persistent or significant disability or incapacity

**Life-threatening* in the definition of a serious adverse event refers to an event in which the patient was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it were more severe.

SAEs should be reported to the Clinical Trials Unit within 7 days. The report should include an assessment of causality by the Principal Investigator at each site (see section 14.6.2). The Chief Investigator will be responsible

for the prompt notification of findings that could adversely affect the health of patients or impact on the conduct of the trial. Notification of confirmed unexpected and related SAEs will be to the Sponsor, the Research Ethics Committee and the Data and Safety Monitoring Committee (DSMC).

14.5. Unexpected Non-Serious Adverse Events

Unexpected non-serious adverse events should be evaluated by the Principal Investigator or research nurse. This should include an assessment of causality (see section 14.6.2) and intensity (see section 14.6.1) and reports made within 14 days. The Clinical Trials Unit will keep detailed records of all unexpected adverse events reported. Reports will be reviewed by the Chief Investigator to consider intensity, causality and expectedness. As appropriate these will be reported to the sponsor, the DSMC and the Ethics Committee.

14.6. Reporting unexpected adverse events

Investigators will make their reports of all unexpected adverse events, whether serious or not, to the Clinical Trials Unit, London School of Hygiene and Tropical Medicine.

14.6.1. Assessment of intensity

Mild: The patient is aware of the event or symptom, but the event or symptom is easily tolerated.

Moderate: The patient experiences sufficient discomfort to interfere with or reduce his or her usual level of activity.

Severe: Significant impairment of functioning; the patient is unable to carry out usual activities and/or the patient's life is at risk from the event.

14.6.2. Assessment of causality

Probable: A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the adverse event and the RIC procedure.

Possible: A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the adverse event and the RIC procedure.

Unlikely: A causal relationship is improbable and another documented cause of the adverse event is most plausible.

Unrelated: A causal relationship can definitely be excluded and another documented cause of the adverse event is most plausible.

15. Withdrawal of patients

15.1. Criteria for withdrawal from the trial

A patient may decide to withdraw from the trial at any time without prejudice to their future care. Withdrawal will be uncommon, because of the non-invasive nature of the planned intervention and the follow up which will be integrated within routine clinical care wherever possible. We have allowed in our sample size calculation for a non-compliance and drop out rate of up to 15% although it is expected to be lower than this.

15.2. Follow up of patients withdrawing from the trial

Patients who are randomised but withdraw before the intervention will undergo standard clinical care according to local protocols. If patients undergo the intervention but subsequently withdraw, they will undergo standard clinical care. Patients will be encouraged to allow data and samples that have been collected before withdrawal to be used in the analyses. However, if consent to use data/samples is also withdrawn, then these will be discarded. Patients withdrawing from the trial will continue to be followed up by their local team. There should be no need for further follow up from the research team.

15.3. Reporting withdrawal of patients

The Clinical Trials Unit at LSHTM should be informed by email if a patient has withdrawn from the trial. A withdrawal from will be completed on the trial eCRF.

16. Statistics

16.1. Trial statistician

Statistical analysis will be coordinated from the Clinical Trials Unit at London School of Hygiene and Tropical Medicine.

16.2. Statistical analysis

A detailed statistical analysis plan will be produced prior to unblinding of any data. The primary analysis will be a comparison of the cardiac death or HHF event rate one year after randomisation between the RIC and sham RIC arms of the trial amongst all STEMI patients. Hazard ratios and confidence intervals will be calculated using Cox proportional hazards modelling and Kaplan-Meier curves will be produced. In addition risk differences at one year will also be calculated together with 95% confidence intervals. The results for the individual components of the primary endpoint will also be presented together with other time to event secondary endpoints such as cardiac death or HHF at 30 days. Differences in means (continuous variables) together with 95% confidence intervals will be calculated using linear regression models and analysis of covariance techniques where appropriate. The primary analysis will be performed on an intention to treat basis i.e. by including all patients where possible according to the group to which they were randomised irrespective of whether they received the intervention as allocated. A secondary per protocol analysis will be undertaken including only patients who receive the allocated intervention as intended.

16.3. Planned subgroup analysis

We plan to undertake a limited number of pre specified subgroup analyses: these will be expected to include diabetes, LAD vs non-LAD STEMI, TIMI flow and time of onset of chest pain to PPCI. The subgroup analyses will be detailed in the statistical analysis plan.

16.4. Procedure to account for missing or spurious data

All patients randomised to the trial will be analysed on an intention to treat basis. Data will be validated and the data analysis will take appropriate account of missing values. This process will be detailed in the statistical analysis plan.

17. Data handling and record keeping

Data will be entered onto an online database and stored securely on Rackspace servers; http://www.rackspace.co.uk and managed by Sealed Envelope™. Data will be kept for 15 years following completion of the trial. The data controller for the trial is the Chief Investigator (UCLH are the data controller's organisation) and the data processor is London School of Hygiene and Tropical Medicine.

18. Insurance

All recruiting centres will be covered by NHS indemnity for negligent harm providing researchers hold a contract of employment with the NHS, including honorary contracts held by academic staff. Medical co-investigators will also be covered by their own medical defence insurance for non-negligent harm.

18.1. Master Indemnity Agreement

The CellAegis autoRIC™ devices are covered by CellAegis for public and product indemnity. CellAegis is registered with the Department of Health under the Master Indemnity Agreement (MIA) reference number IFA2312.

19. Publications policy

It is our intention to disseminate the results of the trial as widely as possible. This is likely to be through a publication in a peer reviewed journal, and through presentations at National and International Cardiology conferences. Publications will follow the CONSORT guidelines. Authorship will follow international guidelines.

20. Expected value of the results

There is an urgent need to improve clinical outcomes in STEMI patients undergoing PPCI. If ERIC-PPCI demonstrates reduced major adverse cardiac events at 12 months in patients treated with RIC at the time of PPCI, there is the potential to change the current management of PPCI patients, to a non-invasive, non-pharmacological, and cost effective therapeutic strategy with benefits in both patient survival and for the prevention of heart failure.

21. Trial organisation

21.1. Trial Steering Committee (TSC)

The TSC will meet every 6 months. The TSC will be responsible for drafting the final report and submission for publication.

Dr Rob Henderson - chair (Independent Interventional Cardiologist)

Prof Derek J Hausenloy (Chief Investigator)

Mr Tim Clayton (Co-Principal Investigator/Senior Medical Statistician with CTU)

Prof Derek Yellon (Co-Principal Investigator)

Dr Rod Stables (Independent Interventional Cardiologist)

Prof Simon Redwood (Co-applicant/Interventional Cardiologist)

Prof Michael Marber (Independent Cardiologist)

Mrs Rosemary Knight (Senior Trial Manager)

Prof Rajesh Kharbanda (Co-Principal Investigator/Interventional Cardiologist)

Mr Paul Hambley (previous PPCI patient)

Mr Alan Berry (previous PPCI patient)

Prof Hans Erik Botker (Interventional Cardiologist and Chief Investigator for Danish CONDI 2 trial)

Observers:

Dr Shannon Amoils (BHF representative)

Ms Tabitha Kavoi (Sponsor representative)

Mr Richard Evans (Trial Manager)

21.2. Project Management Group (PMG)

Prof Derek J Hausenloy (Chief Investigator)

Prof Rajesh Kharbanda (Co-Principal Investigator/Interventional Cardiologist)

Prof Derek Yellon (Co-Principal Investigator)

Mr Tim Clayton (Co-Principal Investigator/Senior Medical Statistician)

Mr Richard Evans (Trial Manager)

Mr Matthew Dodd (Data Manager)

Mrs Rosemary Knight (Senior Trial Manager)

Dr Manish Ramlall (Clinical Research Fellow)

21.3. Data Safety and Monitoring Committee (DSMC)

Prof Colin Berry - **Chair** (independent interventional cardiologist)

Prof Tom Meade (Emeritus Professor of Epidemiology)

Dr Andrew Copas (independent statistician)

Dr Jennifer Nicholas (unblinded statistician at the CTU) will support the DSMC

The DSMC will meet periodically to determine whether there are any unforeseen effects of RIC.

21.4. Endpoint Validation Committee (EVC)

The EVC will meet periodically to validate and adjudicate primary endpoints.

Membership to be decided.

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23. Appendix 1 – CMR protocol

a) Siemens or Philips 1.5 T scanners

b) Transverse half Fourier acquisition single-shot turbo spin-echo sequences for extracardiac anatomical images.

c) Multiplanar balanced steady-state free precession (voxel size, 1.3 x 1.3 x 8 mm3) cine sequences for wall motion abnormalities volumetric analysis.

d) T2 maps acquired from three T2-weighted images at different T2 preparation time (0 ms, 24 ms, and 55 ms, respectively; repetition time = 3 x R-R, voxel 1.9 x 1.9x 6 mm3; motion correction and fitting should then be performed as previously described to obtain the colored T2 maps).

e) Segmented two-dimensional inversion-recovery turbo fast low-angle shot late gadolinium-enhanced (LGE) sequences at 10 - 15 minutes after contrast agent injection (voxel size, 1.3 x 1.3 x 8 mm3).

f) All images to be acquired in breath-hold and to be ECG-triggered.

g) Matching contiguous short-axis views of the entire left ventricle should be obtained for cines, T2 maps and LGE.

h) The contrast agent, Gadoterate meglumine, (gadolinium-DOTA, marketed as Dotarem, Guerbet S.A., Paris, France) at a dose of 0.1 mmol/kg should be administered as a bolus.

24. Appendix 2 – Glossary

AAR	Area at risk	A comparison of the severity of a coronary artery lesion and the volume of heart muscle tissue (myocardium) it supplies. (See also **APPROACH** and **BARI**)
ACS	Acute coronary syndrome	This refers to a group of symptoms caused by obstructed coronary arteries. The symptoms include- 'crushing chest pains', nausea and sweating. These symptoms usually occur as part of an **MI**.
NSAE	Non-Serious Adverse Event	See **SAE**
AF	Atrial fibrillation	A common irregular heartbeat caused by the top chambers in the heart (the atriums) quivering (fibrillating) This rhythm is often the cause of 'palpitations'.
APPROACH	Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease Jeopardy Score	A myocardial jeopardy score used to estimate the amount of myocardium at risk based on the severity of the coronary artery lesion and the volume of myocardium it supplies. (See also **BARI**)
AUC	Area under the curve	Used to calculate release of enzymes (for example Troponin) over time. (See **hsTropT**)
BARI	Bypass Angioplasty Revascularisation Investigation Jeopardy Score	A myocardial jeopardy score used to estimate the amount of myocardium at risk based on the severity of the coronary artery lesion and the volume of myocardium it supplies. (See also **APPROACH**)
BHF	British Heart Foundation	A major funder and authority in cardiovascular research, education and care, and relies predominantly on voluntary donations to meet its aims. In order to increase income and maximise the impact of its work, it also works with other organisations to combat premature death and disability from cardiovascular disease.
Call to balloon time		The time taken from the phone call reporting the heart attack to the start of the angioplasty
CABG	Coronary Artery Bypass Graft	A surgical procedure performed to relieve angina and reduce the risk of death from coronary artery disease. Arteries or veins from elsewhere in the patient's body are grafted to the coronary arteries to bypass atherosclerotic narrowings and improve the blood supply to the myocardium (heart muscle).
CK-MB	Creatinine Kinase	A blood test that measures the presence of cardiac enzymes. These act as markers that can assist in the diagnosis of a heart attack. (See **hs Trop-T**)
CMR	Cardiac MRI	See **MRI**

CONDI2		The Danish arm of the trial. Data will be combined from ERIC-PPCI and CONDI 2 in the analysis.
CRF	Case Report Form	A specialised form (either on paper, or electronic when it's sometimes called an eCRF) used to collect clinical data for a trial or a study.
CTU	Clinical Trials Unit	A specialised research unit which designs, coordinates and analyses clinical trials and other studies.
DSMC	Data Safety and Monitoring Committee	An independent group of experts formed to monitor patient safety and treatment efficacy data while a clinical trial is ongoing.
ECG	Electrocardiogram	A test that records the electric activity of your heart. (ST elevation/depression, T wave, QRS complex- these terms represent aspects of an ECG reading).
EF	Ejection fraction	See **LVEF**
eGFR	Estimated glomerular filtration rate	This is a test to see how well the kidneys are working. It estimates how much blood is filtered by the kidneys over a given period of time.
HHF	Hospitalisation for heart failure	An admission of longer than 24 hours for heart failure. Heart failure is a health condition in which the heart has a reduced ability to pump blood to the body.
hs Trop-T	High sensitivity Troponin-T	A blood test that measures the presence of cardiac enzymes. These act as markers that can assist in the diagnosis of a heart attack. (see **CK-MB**)
IHD	Ischaemic heart disease	Ischaemia is the restriction in blood supply to tissues, resulting in reduced oxygen and glucose supply affecting the cells, causing pain.
IRI	Ischaemia reperfusion injury	the tissue damage caused when tissue experiences a period of ischaemia (or lack of oxygen) and subsequently blood supply returns to the tissue.
LAD	Left anterior descending	One of the arteries of the heart
LBBB	Left bundle branch block	A cardiac contraction condition where activation of the left ventricle is delayed, causing the left ventricle to contract later than the right ventricle. This may require treatment with a pacemaker.
LGE	Late gadolinium enhancement	See **MRI**
LSHTM	London School of Hygiene and Tropical Medicine	The **clinical trial unit** coordinating the ERIC-PPCI trial is based at LSHTM.
LV	left ventricle / left ventricular	Along with the right ventricle, one of the two large chambers that collect and expel blood in the heart.
LVEF	left ventricular ejection fraction	Often given as a percentage, it is the volumetric fraction of blood pumped out of the left ventricle in the heart with each heartbeat.

MI	Myocardial infarction	Or 'Heart attack'. An Interruption of blood supply caused by a blockage in the blood vessels to the heart leading to cell or tissue death (infarction). Sometimes referred to as NSTEMI or **STEMI**.
MRI	Magnetic resonance imaging	A medical imaging technique used in radiology to visualise internal structures in the body. (LGE- Late gadolinium-enhanced images- A more advanced MRI).
Myocardium		The myocardium is the muscle tissue of the heart, and forms a thick middle layer between the outer epicardium layer and the inner endocardium layer.
NIHR	National Institute for Health Research	The NIHR is the health research arm of the NHS.
NYHA class	New York Heart Association	A simple way of classifying the extent of heart failure (see definition) using physical activity, chest pain and breathlessness as a measure. See **CCS**.
OMT	Optimal Medical Therapy	This includes the best medication (tablets) that are currently available for heart failure, at doses that are individually tailored. This strategy often also involves insertion of a special type of pacemaker (called a biventricular pacemaker, which may also function as an Implantable Cardioverter Defibrillator).
PI	Principal Investigator	The doctor leading the trial at the site level. Each site has a principal investigator who will delegate roles and responsibilities to other staff using a delegation log.
PIS	Patient Information Sheet	A leaflet given to the patient which explains the trial and their involvement in it in lay language.
PPCI	Primary percutaneous coronary intervention	This procedure is used to treat the narrowed coronary arteries of the heart. A small tube is inserted in the groin or wrist and advanced to the heart. Small balloons and stents are used to open up the narrowings and improve blood flow to the heart muscle. This is sometime also known as Primary Angioplasty.

RIC	Remote ischaemic conditioning	RIC describes the process of applying cycles of limited blood flow (ischaemia) and reinstated blood flow (reperfusion) to an organ or tissue as a protection mechanism for other organs. The full extent and mechanism of this protection is unclear. However there is evidence from previous extensive work on patients that there is a pathway linking the pre conditioned organ or tissue (in the case of ERIC-PPCI the upper arm) to the heart.
SAE	Serious Adverse Event	Any event such as an illness or an accident that results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.
SBP	Systolic Blood Pressure	During each heartbeat, blood pressure varies between a maximum (systolic) and a minimum (diastolic) pressure.
SD	Standard deviation	Measures the amount of variation from the average.
SPECT	Single-photon emission computed tomography	An imaging technique that produces three dimensional images of functional processes in the body.
SSFP	Steady-state free precession imaging	A magnetic resonance imaging (**MRI**) technique which uses steady states of magnetisations.
STEMI	ST elevated myocardial infarction	ST elevation refers to a finding on an **ECG**, wherein the trace in the ST segment is abnormally high above the isoelectric line.
TIMI Perfusion Grade (Blush)	Thrombolysis in myocardial infarction perfusion grade	A technique to assess myocardial infusion in the capillary bed on a coronary angiogram
TIMI flow grade	Thrombolysis in myocardial infarction flow grade	A measure to assess epicardial coronary blood flow
UK NIAP	UK National Infarct Angioplasty Project	A joint project set up by the British Cardiac Society and the Department of Health to test the feasibility of implementing a countrywide angioplasty service to treat cases of acute myocardial infarction in England.

25. Appendix 3 – EuroQol EQ-5D-5L

ERIC-PPCI

Effect of Remote Ischaemic Conditioning on clinical outcomes in ST segment elevation myocardial infarction patients undergoing Primary Percutaneous Coronary Intervention

Health Questionnaire

English version for the UK

ERIC-PPCI Trial Number:	E P ☐☐☐☐☐
Date of Birth:	d d m m y y y y

EQ-5D-5L

ERIC-PPCI Trial Number: **E P** | | | | | | |

Date completed | d | d | m | m | y | y | y | y |

Under each heading, please tick the **ONE** box that best describes your health **TODAY**

Mobility

I have no problems in walking about	
I have slight problems in walking about	
I have moderate problems in walking about	
I have severe problems in walking about	
I am unable to walk about	

Self-Care

I have no problems washing or dressing myself	
I have slight problems washing or dressing myself	
I have moderate problems washing or dressing myself	
I have severe problems washing or dressing myself	
I am unable to wash or dress myself	

Usual Activities (e.g. work, study, housework, family or leisure activities)

I have no problems doing my usual activities	
I have slight problems doing my usual activities	
I have moderate problems doing my usual activities	
I have severe problems doing my usual activities	
I am unable to do my usual activities	

Pain/Discomfort

I have no pain or discomfort	
I have slight pain or discomfort	
I have moderate pain or discomfort	
I have severe pain or discomfort	
I have extreme pain or discomfort	

Anxiety / Depression

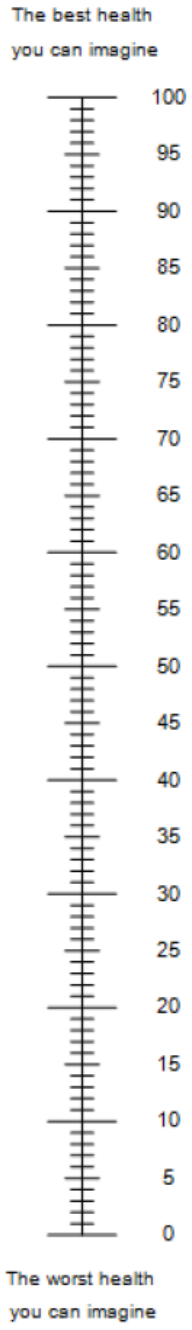
I am not anxious or depressed	
I am slightly anxious or depressed	
I am moderately anxious or depressed	
I am severely anxious or depressed	
I am extremely anxious or depressed	

EQ-5D-5L

ERIC-PPCI Trial Number: **E P** | | | | | |

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the **best** health you can imagine.
- 0 means the **worst** health you could imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = | |



Appendix 4: Additional Substudies

Coronary Physiology substudy

Background and rationale

The assessment of the efficacy of reperfusion at the time of PPCI is challenging. Angiography and ST-resolution alone are good but imperfect indices. There is increasing data to support the use of coronary physiology indices measured at the time of PPCI as a surrogate index to measure the efficacy of reperfusion and provide insights into the effects on the microcirculation. Thus, the index of microcirculatory resistance (IMR) relates to biochemical and MRI infarct size, and an IMR value of >40 units (observed in about 1/3 PPCI patients) at the completion of PPCI is associated with adverse outcome. Recent intervention studies have adopted IMR as primary endpoint to test the effects of drugs or aspiration, although the clinical significance of this is uncertain. Our own group has been studying the changes in coronary physiology indices at the time of PPCI after aspiration and before coronary stenting. In addition we have used the opportunity to sample coronary sinus, aortic, culprit and venous blood for biomarker studies.

Hypothesis

1. RIC has beneficial effects on IMR at the completion of PPCI compared with control
2. RIC improves the response of the microcirculation at the time of PPCI to coronary stenting
3. IMR relates to CMR imaging indices in an interventional trial
4. Identification of circulating marker or effluent from the myocardium induced by RIC

Method

Coronary flow and pressure measurements are made using a coronary pressure/temperature sensitive guidewire, using established methods in routine use in clinical PCI procedures. The pressure wire is interchangeable with the coronary guide wire used in all PCI procedures, so is incorporated in to the procedure. Measurements that assess the integrity of the small blood vessels of the heart are made during the PPCI procedure, once the artery is opened and blood flow has been restored to the heart, and after implantation of a coronary stent Saline is flushed through the guide catheter to measure coronary flow using the transit time calculation. Adenosine is used as in standard clinical practice to increase blood flow. [1]

Sample Size

There are no published trials in this area and so sample size calculations have been based on similar patient groups and published literature: In the TIME trial mean IMR in an ACS cohort is 30 (SD 20). An effect size of 30% reduction in IMR is proposed, and allowing for dropout a sample size of 152 patients has been calculated at 80% power, 5% significance. The PATA STEMI study has an IMR reduction from 38U with a 26% effect size, and 128 patients. A study comparing aspiration and abciximab powered to show an almost 40% reduction in IMR from 34 to 20 calculated a sample size of 40. Published data suggest that the mean IMR after PPCI is 38 with an SD of 30. We would propose an effect size of 30% reduction in the treatment group and at 80% power with significance 5% the sample size would be 168 patients (84 per group). Allowing for dropout 180 patients will be required.

Feasibility

Coronary physiology and IMR measures are practical and feasible in STEMI PPCI and we have extensive experience of these protocols in John Radcliffe Hospital Oxford as part of an ongoing OxAMI study, which is an observational cohort study. [2-6]

John Radcliffe Hospital, Oxford will be the lead centre. Additional selected sites will be invited to participate in this substudy.

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Thrombosis substudy

Background

The pathogenesis of acute coronary syndromes (ACS) involves thrombosis on a background of plaque rupture and erosion, which can manifest in ST-elevation MI (STEMI). The risk of thrombosis is determined by the balance between the prothrombotic forces in blood and the capacity of the innate endogenous thrombolytic system to naturally dissolve any thrombus. Until recently, endogenous thrombolysis has been difficult to measure. Impaired thrombolytic status has been shown in NSTEMI, end-stage renal disease, stroke, and diabetes; and is increasingly recognised as a risk factor for arterial thrombosis. Effectiveness of endogenous thrombolysis is determined by thrombus properties (clot strength, determined by fibrin density, pore size and size of fibrin strands) and the rate of fibrinolysis, determined mainly by release of t-PA from the endothelium and PAI-1 from activated platelets. The exact mechanism through which ischaemic preconditioning improves outcomes in patients with STEMI is still not fully understood. In dogs, ischaemic preconditioning has been accompanied by down-regulation of platelet-fibrinogen binding and formation of neutrophil-platelet aggregates (1). In patients, platelet reactivity was reduced after repetitive, compared to single cycle exercise (2), whilst marked systemic platelet activation has been demonstrated in ACS (3) or acute limb ischemia (4). In patients undergoing radiofrequency ablation of atrial fibrillation, RIC reduced platelet activation and reactivity (5). In patients with subarachnoid hemorrhage, RIC prolonged the PT and INR (6). Activated platelets play an important role in the process of myocardial ischemia-reperfusion injury, and platelet-derived P-selectin is a critical mediator. In patients with coronary artery disease (7), RIC prior to exercise stress testing reduced ADP-stimulated platelet aggregation. However, the potential clinical benefit of any of these findings remains to be seen. Our pilot data on thrombotic status in PPCI: Data on 80 STEMI patients has yielded promising results. Rapid endogenous fibrinolysis was related to spontaneous STsegment resolution, TIMI 3 flow at presentation, and uneventful outcomes. Impaired endogenous lysis was associated with TIMI 0 flow and MACE within 30 days.

Hypothesis

Platelet activation and impaired endogenous thrombolysis are critical determinants of outcome in patients with STEMI undergoing PPCI. RIC reduces ischaemic insult and improves outcome, and this is at least in part, through favourable effects on thrombotic status.

Methods

All patients participating in ERIC-PPCI that are recruited at Lister Hospital, Stevenage will be enrolled.

Blood samples

Venous blood samples (20ml) will be taken at presentation, 1 day post and 2-3 days post-STEMI and at the 6-8 week follow-up visit, for immediate point-of care thrombotic assessment, and the remainder will be stored in an accredited lab for later assessment of thrombogenesis and fibrinolysis. Samples will be taken by trained cath lab nurses and doctors already familiar with this protocol.

Assessment of Thrombotic Status

Thrombotic status will be assessed using the point-of-care Global Thrombosis Test (GTT, Thromboquest Ltd., UK) and tests of thrombogenesis and fibrinolysis using micro-titre based assays on fresh frozen plasma (FFP). The GTT is a relatively novel point-of-care test, utilising native, nonanticoagulated whole blood, without external agonists, under high shear stress conditions, akin to that in a stenosed artery. It assesses platelet aggregation (time to occlusive thrombus formation; occlusion time (OT) and endogenous thrombolytic status (time to endogenous lysis of thrombus formed in the first phase of the test; lysis time(LT)) [10,11,21].

Other Blood Tests

At each timepoint, a measurement of coagulation (fibrinogen, INR) and inflammation (high sensitivity C-reactive protein). Blood will also be spun and stored for future analysis as determined by study results.

Follow-up

Follow up will be the same as the ERIC-PPCI protocol, however all 6-8 week follow-up will be conducted at the outpatient clinic.

Handling of results

Patients receiving RIC or sham RIC, will be compared with respect to thrombotic status pre and post-PPCI, as well as the absolute change in thrombotic status pre and post intervention, and differences in ECG ST-resolution and clinical outcomes.

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Myosin C Substudy

Background

Cardiovascular disease is one of the leading causes of death in the United Kingdom (British Heart Foundation statistics, 2012). Early diagnosis and treatment of acute myocardial infarction helps reduce infarct size and long-term mortality. Current guidelines advocate the use of cardiac troponins I and T as the biomarkers of choice to help diagnose and identify patients at high risk who will benefit the most from early invasive therapy. However, their slow release hinders prompt diagnosis and treatment. New generation high-sensitivity assays have sensitivities of less than 85% for chest pain onset within 3 hours. A varying range of positive predictive values (42–83%) also produces many false positives[1, 2]. Furthermore, persistently raised enzyme levels even a week after the index event makes the diagnosis of re-infarction challenging.

Jacquet & al. have identified a novel biomarker of myocardial injury, cardiac myosin binding protein-C (cMyBP-C), which can potentially address some of the pitfalls of troponin assays[3]. A study by Kuster & al. demonstrated that cMyBP-C levels were detectable 30 minutes following ST-segment elevation myocardial infarction (STEMI) in pigs[4]. The levels peaked after 6 hours and returned to baseline within 12 hours. Interestingly, the study also demonstrated no correlation between cMyBP-C and troponin levels in a cohort of non-ST-segment elevation myocardial infarction (NSTEMI) patients. There are currently no studies which have studied the release kinetics of cMyBP-C in STEMI patients and its correlation with infarct size.

Hypothesis

Elevations in the cMyBP-C levels correlate with infarct size as measured by high-sensitivity Troponin T (hsTrop T) levels and mass of late-gadolinium enhancement on cardiac magnetic resonance imaging (MRI).

Methods

From each patient, a blood sample will be taken at each of the 5 time-points (0, 6, 12, 24 and 48 hours following PPCI procedure). Each blood sample will be processed (centrifuged to separate serum) and stored (at -20°C or below).

This will be a single-centre substudy based at the Barts Heart Centre hospital.

Analysis

An optimised sandwich immunoassay on the Singulex Erenna platform using two antibodies (1A4 and 3H8) is used as follows:

Magnetic microparticles (MPs, Singulex) for capture are prepared by binding 25µg of mouse monoclonal antibodies (1A4) per mg of MPs. The coated MPs are diluted in assay buffer (Singulex proprietary mix with custom 450mM NaCl and 0.5% Triton X-100) to 100µg/mL. Serum, plasma or analyte (recombinant C0C2 domain of cMyC (12)) are diluted 1:1 in an equal volume of standard diluent (Singulex) and 100µL added per well of a 96-well assay plate. Samples or standards are then exposed to 100µL of coated MPs and agitated for 2 hours at 25°C. MPs are retained via a magnetic bed with unbound material removed in a single wash step. Fluorescently-labelled mouse monoclonal (3H8) detection antibody is diluted in assay buffer (Singulex) to 100ng/mL. To each well, 20µL of detection antibody is added and the MPs agitated for 1 hour at 25°C, retained via a magnetic bed and then washed 4 times to remove any unbound detection reagent. The MPs are then transferred to a new plate and all buffer is aspirated. The MPs are then exposed to 20µL/well of elution buffer B (Singulex) for 5 minutes at 25°C before transfer to a 384-well plate containing 10µL/well of neutralization buffer D (Singulex). Fluorescent label is then detected by single molecule counting using the Erenna system (Singulex) with a dwell time of 60s per well. Three signal outputs can be obtained from the Erenna System: Detected Events (DEs; low end signal), Event Photons (EPs; low end and higher end signal), and Total Photons (TPs; high end signal). The LLoQ is 1.2ng/L and the calculated LoD is 0.4ng/L; Intra- and inter-assay precision average 11±3% and 13±3%, respectively.

Sample size

There are currently no studies which have identified the correlation between cMyBP-C and infarct size in STEMI patients. Based on unpublished work done by our group and a recent study by Reinstadler & al., the correlation coefficients between hsTrop T levels and infarct size as measured by cardiac MRI are 0.64 and 0.70 for 24-hour area-under-curve and peak levels respectively. To calculate the required sample size, we assume that the probability for rejecting the null hypothesis (α) is equal to 0.05 and the probability of failing to reject the null hypothesis under the alternative hypothesis (β) is equal to 0.20. We also assume that cMyBP-C levels will have at least equal or similar correlation with infarct size. Using a lower correlation coefficient of 0.6 and a missing data rate of 25%, a sample size of 24 will be required.

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