Effect of remote ischaemic conditioning on platelet reactivity and endogenous fibrinolysis in ST-elevation myocardial infarction- a substudy of the CONDI-2/ERIC-PPCI randomised controlled trial

Short title: Effect of remote ischaemic preconditioning on platelet reactivity and fibrinolysis in PPCI

Prof. Diana A Gorog MD, PhD, FRCP[1,2,3] Dr Mohamed Farag MB, BS, PhD [2,4], Dr Nikolaos Spinthakis MB BS, MRCP[2,3], Prof. Derek M Yellon DSc[5], Prof. Hans Erik Bøtker DMSci[6], Prof. Rajesh K. Kharbanda MB, BCh, PhD[7], Prof. Derek J Hausenloy MBChB PhD FRCP[8]

1. National Heart & Lung Institute, Imperial College, London, United Kingdom
2. Postgraduate Medical School, University of Hertfordshire, Hertfordshire, United Kingdom
3. Department of Cardiology, East and North Hertfordshire NHS Trust, Hertfordshire, United Kingdom
4. Cardiology Department, Royal Papworth Hospital NHS Foundation Trust, Papworth Everard, Cambridge, United Kingdom
5. The Hatter Cardiovascular Institute, University College London, London, United Kingdom
6. Aarhus University Hospital, Department of Cardiology, Aarhus N, Denmark.
7. Oxford Heart Centre, Oxford University Hospitals NHS Trust, Oxford, United Kingdom; Department of Cardiovascular Medicine, University of Oxford, Oxford, United Kingdom.
8. The Hatter Cardiovascular Institute, University College London, London, UK;; Cardiovascular & Metabolic Disorders Program, Duke-National University of Singapore Medical School, Singapore; National Heart Research Institute Singapore, National Heart Centre, Singapore; Yong Loo Lin School of Medicine, National University Singapore, Singapore; Cardiovascular Research Center, College of Medical and Health Sciences, Asia University, Taiwan; Tecnologico de Monterrey, Centro de Biotecnologia-FEMSA, Nuevo Leon, Mexico.

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Correspondence:

Prof. Diana A Gorog MB BS, MD, PhD, FRCP
National Heart and Lung Institute
Dovehouse Street
London SW3 6LR
United Kingdom
Tel +44 (0)207 034 8934; Fax +44 (0)207 0348935
d.gorog@imperial.ac.uk

Abstract

Background: Remote ischaemic conditioning (RIC) has been shown to reduce myocardial infarct size in animal models of myocardial infarction. Platelet thrombus formation is a critical determinant of outcome in ST-segment elevation myocardial infarction (STEMI). Whether the beneficial effects of RIC are related to thrombotic parameters is unclear.

Methods and Results: In a ~~pre-specified~~ substudy of the Effect of Remote Ischaemic Conditioning on clinical outcomes in STEMI patients undergoing Primary Percutaneous Coronary Intervention (ERIC-PPCI) trial, we assessed the effect of RIC on thrombotic status. Patients presenting with STEMI were randomised to immediate RIC consisting of an automated autoRIC^{TM} cuff on the upper arm inflated to 200mmHg for 5 minutes and deflated for 5 minutes for 4 cycles (n=53) or sham (n=47). Venous blood was tested at presentation, discharge (48 h) and 6-8 weeks, to assess platelet reactivity, coagulation and endogenous fibrinolysis using the Global Thrombosis Test and thromboelastography (TEG). Baseline thrombotic status was similar in the 2 groups. At discharge, there was some evidence that the time to *in vitro* thrombotic occlusion under high shear stress was longer with RIC compared to sham (454±105s vs. 403±105s; mean difference 50.1s; 95% confidence interval [CI] 93.7-6.4, P=0.025), but this was no longer apparent at 6-8 weeks. There was no difference in clot formation or endogenous fibrinolysis between the study arms at any time-point.

Conclusion: RIC may reduce platelet reactivity in the first 48h post-STEMI. Further research is needed to delineate mechanisms through which RIC may reduce platelet reactivity, and whether it may improve outcomes in patients with persistent high on-treatment platelet reactivity.

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Abbreviations

ADP = adenosine diphosphate

DAPT = dual antiplatelet medication

GTT = Global Thrombosis Test

IPC = ischaemic preconditioning

IR = ischaemia-reperfusion

IRI = ischaemia-reperfusion injury

LT = lysis time

OT = occlusion time

PPCI = primary percutaneous coronary intervention

RIC = remote ischaemic preconditioning

STEMI = ST-segment elevation myocardial infarction

TEG = thromboelastography

Introduction

The cause of ST-segment elevation myocardial infarction (STEMI) is most commonly the disruption of a coronary atheromatous plaque, leading to local thrombosis, and culminating in arterial occlusion. The outcome of such a prothrombotic stimulus is determined by the magnitude of the thrombotic response, balanced against the effectiveness of the endogenous fibrinolytic enzymes in overcoming lasting vessel occlusion.[1] Treatment of STEMI patients with primary percutaneous coronary intervention (PPCI) aims to rapidly restore coronary flow, improve myocardial salvage and reduce infarct size. However, reperfusion has also been associated with consequent downstream myocardial reperfusion injury, which may further compound the deleterious effects of the antecedent period of ischaemia.[2–4] Measures to ameliorate the thrombotic response and reduce ischaemic-reperfusion injury (IRI) have been proposed to reduce infarct size.[1,3–5]

Ischaemic preconditioning (IPC) refers to the ability of brief, cyclic periods of ischaemia and reperfusion (IR) to render the myocardium more resistant to a subsequent ischaemic insult. In animal models, IPC has been shown to reduce infarct size and to enhance recovery of contractile function of the myocardial region at risk.[6] Remote ischaemic conditioning (RIC) involves the application of one or more brief cycles of IR to a "remote" organ (such as the arm or leg) and in animal models, has been shown to reduce infarct size and IRI.[7–9]

Application of RIC in humans by repeated inflation and deflation of a blood pressure cuff on the upper arm has been shown to reduce the extent of perioperative myocardial injury in patients undergoing cardiac surgery in smaller studies,[10] although it did not improve clinical outcomes in large studies.[11,12] Compared to standard care, the use of RIC in patients undergoing PPCI has been associated with reduction in myocardial injury and increased myocardial salvage, without definitive reduction in infarct size or improvement in survival.[13,14]

The exact mechanism through which RIC potentially confers cardioprotection in STEMI is still not fully understood.[15,16] Proposed mechanisms include generation of an endogenous substance such as adenosine, bradykinin or other factor, which activates a neural pathway; mediation by an endogenous substance generated in the remote organ which enters the blood stream to affect cardioprotection; or through a systemic protective response, suppressing inflammation and apoptosis.[15,16] Additionally, IPC has been linked to favourable effects on thrombotic markers. In a canine model, IPC was accompanied by down-regulation of platelet-fibrinogen binding and formation of neutrophil-platelet aggregates.[17] In stable CAD, remote ischaemia was shown to induce protection against an exercise-related increase in platelet reactivity[18] and reduced ADP-stimulated platelet aggregation. In patients undergoing radiofrequency ablation for atrial fibrillation, RIC reduced platelet activation and platelet reactivity.[19] Since platelet reactivity, activation of coagulation and endogenous fibrinolytic pathways are important drivers and determinants of the outcome of myocardial infarction,[20] and may play a role in IRI,[21] we hypothesised that the benefit of RIC in STEMI may be mediated through anti-thrombotic effects. The aim of this study was to determine whether RIC improves thrombotic status in patients with STEMI undergoing PPCI.

Methods

Study design and population

We undertook a substudy of the Effect of Remote Ischaemic Conditioning on clinical outcomes in ST-segment elevation myocardial infarction patients undergoing Primary Percutaneous Coronary Intervention (ERIC-PPCI) multicentre, randomised, single-blind, placebo-controlled clinical trial (ClinicalTrials.gov NCT02342522).[22] Patients with chest pain and suspected ST-segment elevation on the electrocardiogram (ECG) were screened for possible inclusion. Patients were included if they were older than 18 years of age, had ST-segment elevation on ECG, were eligible for PPCI and gave consent. Exclusion criteria were previous coronary artery bypass graft surgery, myocardial infarct within the previous 30 days, left bundle branch block on ECG, treatment with therapeutic hypothermia, conditions precluding use of remote ischaemic conditioning (paresis of upper limb or presence of an arteriovenous shunt), and life expectancy of less than 1 year due to a non-cardiac pathology. All patients recruited to ERIC-PPCI in a single centre at the Lister Hospital, East & North Hertfordshire NHS Trust, were included in the substudy. The study was approved by the National Research Ethics Service and was conducted in accordance with the principles of Good Clinical Practice and the trial conformed to the principles outlined in the Declaration of Helsinki. All patients provided initial verbal assent before randomisation, which was followed by written informed consent.

Trial treatment protocol

The trial protocol and main clinical results have been previously published.[22,23] In brief, patients were randomised in a 1:1 ratio to active treatment with RIC or control treatment with sham RIC (Figure 1). Randomisation was performed via a secure website using random

permuted blocks. Patients randomised to the interventional arm received RIC protocol using the automated AutoRIC cuff device (CellAegis Devices, Toronto, ON, Canada), comprising of four alternating cycles of cuff inflation to 200 mm Hg for 5 min and deflation for 5 min. The control group received a sham simulated RIC. The PPCI procedure was performed according to standard clinical care and PPCI operators and patients were blinded to treatment allocation. Study team members collecting the data and assessing outcomes were masked to treatment allocation.

All patients received 300 mg aspirin orally and 600 mg clopidogrel or 180 mg ticagrelor orally, and standard weight-adjusted heparin intravenously prior to PPCI. Dual antiplatelet therapy was continued in all patients throughout the substudy.

Blood sampling technique

Blood samples were taken at three time points: 1) baseline upon arrival to the cardiac catheterisation laboratory (day 0), prior to heparin or glycoprotein IIb/IIIa inhibitor administration and before PPCI, 2) at clinical stabilisation, just prior to hospital discharge, and 3) at 6-8 weeks follow-up. The first blood samples were taken from a 6-F radial or femoral sheath, after the administration of dual antiplatelet therapy (DAPT) but before treatment with unfractionated heparin. Prior to insertion, the sheaths were flushed with normal saline, avoiding the use of heparinised saline prior to the first blood draw. The second and subsequent blood samples were taken from an antecubital vein using an 18-G butterfly cannula, taking care to avoid prolonged tourniquet time. All samples were taken using a 2-syringe technique, which involved using the first 5 ml blood for routine blood tests, and the subsequent sample for assessment of thrombotic status.

Assessment of global thrombotic status

Global Thrombosis Test (GTT)

The GTT (Thromboquest Ltd., London, UK) assesses both platelet reactivity (occlusion time, OT) and endogenous fibrinolysis (lysis time, LT) from a 4 ml native, non-anticoagulated blood sample. The instrument was positioned in the cardiac catheterisation laboratory. After the blood sample was obtained, it was introduced into the GTT cartridge within 15 seconds of withdrawal and the automated measurement begun. The principle of the GTT has been previously described in detail.[24,25] The instrument assesses firstly the time taken to form an occlusive thrombus under high shear (occlusion time, OT; sec), a marker of platelet reactivity. Shorter OT represents enhanced platelet reactivity. The arrest of flow due to the formation of an occlusive platelet thrombus, is followed by a short stabilisation period, after which the instrument records the time required for spontaneous restart flow due to endogenous thrombolysis of the thrombus formed in the first phase (lysis time, LT; sec). Longer LT represent less effective endogenous fibrinolysis.

Thromboelastography (TEG)

Blood was also tested using the TEG thromboelastograph (TEG 5000 Hemostasis Analyser system, Haemonetics, UK). Two tests were carried out per patient in parallel; whole blood (without the addition of any modifiers) and whole blood plus kaolin (Haemonetics, Watford, UK). Whole blood testing was performed immediately after sampling, whereas whole blood plus kaolin was performed within 4 minutes of sampling. The TEG generates a characteristic curve of thrombus formation and lysis with several indices, and definition of these is shown in Table 1.[26]

Study end-points

The endpoint of the substudy was thrombotic status as measured by GTT and TEG parameters, in the RIC compared to the sham arms, at discharge and at 6-8 weeks. The primary combined endpoint of the main study was cardiac death or hospitalisation for heart failure at 12 months and these results have been published.[22]

Data collection and follow-up

Patient case-notes were checked throughout the course of the index admission, to allow contemporaneous data collection. Patients were followed up at 6-8 weeks in person including final blood draw for thrombotic status assessment.

Statistical analysis

In this pilot, hypothesis-generating substudy, we aimed to compare thrombotic status within groups (between patients on admission and at discharge and follow-up) and between groups (between RIC and sham). For a main trial designed with 90% power and two-sided 5% significance, it is recommended that a pilot trial sample size of at least 20 per treatment arm is needed for estimated small (0.2) standardised effect size,[27] which was speculated from earlier studies.[25] Therefore, a study of 100 patients (50 per treatment arm) was felt to be of sufficient size to produce meaningful results. Data are presented as mean and standard deviation (when normally distributed) or median and inter-quartile range [IQR] (non-normally distributed). Normality was tested using the Shapiro-Wilk test. Differences in thrombotic variables at differing time-points in the group as a whole were assessed using paired-t-tests and Mann-Whitney U test. Difference between RIC and sham groups at any

individual time-point were assessed using ANCOVA. Analyses were performed with Stata version 11.2 (StataCorp, College Station, TX, USA).

Results

Between February 2016 and March 2018, 100 patients with STEMI were enrolled into the substudy, and randomised to RIC (n=53) or sham RIC (n=47) (Supplementary Figure 1). The main ERIC-PPCI study results have already been published.[22] Baseline clinical characteristics are shown in Table 2 and baseline haematological and biochemical profiles in Table 3. There were no patients with atrial fibrillation or patients taking oral anticoagulation included in this substudy. Angiographic, interventional and echocardiographic patient characteristics are shown in Table 4. The RIC and sham groups were well matched for all aforementioned characteristics. In particular, there was no significant difference in either peri-procedural or post-PPCI antithrombotic treatment allocation between the treatment arms.

Global Thrombosis Test (GTT) results

In the whole cohort (n=100), OT increased from baseline to hospital discharge (338±129s vs. 430±107s, p<0.001) and further increased at 6-8 weeks (baseline vs. 6-8 weeks 338±129s vs. 493±132s, p<0.001)(Figure 2A).

Baseline OT was similar in the RIC and sham groups, with mean difference 19.65s (95% confidence interval [CI] 69.41-70.36) (Table 5, Figure 3). However, there was some evidence that OT at hospital discharge was prolonged in RIC group compared to sham (454±105s vs. 403±105s; mean difference 50.1s; 95% CI 6.4-93.7, P = 0.025), but this was less apparent at

6-8 weeks follow-up (538±142s vs. 511±142s, mean difference 27.5s; 95%CI 102.5- 47.5, P=0.818) (Table 5, Figure 3).

Distribution of LT at the prespecified time points is shown in Figure 2-B. There was no evidence for a difference in LT between the two study arms at any of the time points (Figure 4 and Table 5).

Thromboelastography (TEG) results

There was no evidence for a difference in any of the TEG indices using whole blood with or without kaolin between the two study arms at any of the time points, either with respect to coagulation parameters or indices of clot lysis (Table 5).

DISCUSSION

In this small, hypothesis generating substudy, in the group as a whole, OT was higher at discharge compared to admission, presumably reflecting reduction in platelet reactivity, due to onset of action of DAPT. However, although baseline thrombotic status at presentation was similar in patients in both RIC and sham RIC groups, patients receiving RIC exhibited significantly longer OT, representative of reduced platelet reactivity, at the time of hospital discharge compared to patients treated with sham RIC. This is, to our knowledge, the first time that RIC has been linked to reduced occlusive thrombus formation under high-shear stress, in the setting of STEMI in humans.

The encouraging results of this substudy contrast with the neutral results of the main CONDI-2/ERIC-PPCI trial, in which no difference was seen between the RIC and the control groups with respect to the combined primary endpoint of cardiac death or hospitalisation for heart

failure at 12 months (HR 1.10; 95% CI 0.91–1.32; P = 0.32), demonstrating that RIC, applied as an adjunct to PPCI, did not improve clinical outcomes in STEMI patients. The discrepancy between the findings of our small substudy and the main trial may simply be due to the play of chance in a small sample. However, if these results are real, and RIC results in reduced platelet reactivity at 48h post-PPCI, it would not be surprising if this in fact had no effect on outcomes. The reduction in platelet reactivity at 48h may be too late to influence reperfusion and infarct size, or to favourably impact on any reperfusion injury following PPCI. This might indicate that earlier application of such RIC may have improved outcomes, although in the main CONDI-2/ERIC-PPCI trial, there were no differences in clinical outcomes whether RIC was performed in the ambulance or in hospital. Another consideration is that platelet reactivity is a strong determinant of ischaemic outcomes, in particular in the highest risk patients. Although acute stent thrombosis is likely multifactorial in aetiology, it has been been related in part to enhanced platelet reactivity, and so it is possible that a beneficial effect in reducing platelet reactivity could reduce the occurrence of acute stent thrombosis, although there was no signal for this in the main CONDI-2/ERIC-PPCI trial, where the occurrence of myocardial infarction at 30 days was similar in the RIC and sham arms. The CONDI-2/ERIC-PPCI trial excluded many patients with anterior STEMI, since these often exhibit left bundle branch block, and patients with cardiogenic shock who were unable to give consent. Patients with cardiogenic shock are not only at very high cardiovascular risk with 30-50% risk of death or recurrent ischaemic events over the subsequent 30 days, but shock can also limit the effectiveness of orally-administered antithrombotic medications due to delayed drug administration, reduced gastrointestinal blood flow and motility, delayed gastric emptying and gastrointestinal absorption[29]- so these patients may have the most to gain from approaches that reduce platelet reactivity. Since the effect on platelet reactivity was no longer apparent at 6-8 weeks, this may explain the lack of effect on long term ischaemic outcomes.

Whilst current guidelines advocate use of the more potent $P2Y_{12}$ inhibitors ticagrelor and prasugrel in patients with STEMI,[30] this also comes at a greater price of bleeding. Clopidogrel continues to be used in a significant number of ACS patients in high income countries,[31] and also for financial reasons in low income countries.[32] Up to a third of ACS patients demonstrate inadequate platelet inhibition in response to clopidogrel.[33] This is explained in part by polymorphisms in the gene encoding the hepatic enzyme *CYP2C19*, which transforms clopidogrel to its active metabolite, that can result in 5-12% variation in platelet inhibition.[34] There is ethnic variation in the prevalence of the loss-of-function *CYP2C19* 618G>A*2 allele, affecting some 30% of Caucasians and 50% of East Asians.[33] Homozygotes for the *CYP2C19*2* and less common *CYP2C19*3* LoF alleles are poor metabolizers, and heterozygotes are intermediate metabolizers of clopidogrel, with high-on clopidogrel platelet reactivity and increased risk of adverse cardiovascular events, including AMI and stent thrombosis.[35–37] The association of *CYP2C19* genotype with increased cardiovascular risk appears greatest in those undergoing PCI, and the risk is greater in Asians than in whites.[38] Enhancing platelet inhibition with RIC in patients who are receiving clopidogrel may be particularly advantageous in such patients.

Possible mechanisms

A possible mechanism underlying the beneficial effects of RIC is a direct effect on arterial thrombus formation. In humans, marked platelet activation has been demonstrated in patients presenting with acute coronary syndrome[39,40] and platelets have an important role not only in epicardial coronary thrombosis, but also in the pathophysiology of IRI and IPC.[41–43]

The relationship between RIC and platelet activation is less well explored in patients, with most knowledge derived from animal studies and healthy volunteers. In rats, RIC reduced

arterial thrombus formation and embolization under direct visualisation by microscopy following femoral arterial injury[44] and in rodent hearts *ex vivo*, the extent of myocardial injury following IR injury is was directly related to the activation status of platelets, with reduced infarct size in mice treated with platelet-poor plasma.[42] Platelet-derived microparticles may mediate RIC, since platelet microparticles isolated from rats receiving RIC reduced the extent of cerebral infarction when transfused into recipient rats.[45] In dogs subjected to coronary IR injury, IPC attenuated platelet activation and aggregation[17,46] and was abolished by pre-treatment with an adenosine antagonist, linking preconditioning with platelet thrombus formation.[46]

Studies in healthy individuals support the concept that RIC inhibits platelet activation. In healthy volunteers, the increase in the circulating concentration of platelet–monocyte aggregates associated with acute IR injury was abolished by RIC.[47] In normal volunteers, RIC of forearm reduced expression of neutrophil CD11b and platelet–neutrophil complexes.[48] Studies in patients with cardiovascular disease are limited. In patients with stable coronary disease, RIC attenuated platelet activation in response to adenosine diphosphate (ADP) and exercise[18] and in patients with claudication, warm-up (a phenomenon akin to IPC) prior to exercise attenuated the exercise-induced increase in platelet–neutrophil and platelet–leukocyte activation.[49] In patients undergoing ablation for atrial fibrillation, RIPC reduced platelet activation in response to ADP, including the formation of monocyte-platelet aggregates.[19] Other studies found that intermittent upper arm IR reduced platelet activation and aggregation in response to ADP in patients with stable angina undergoing angiography or elective angioplasty.[50]

If the effect of RIC is marked in animals, in healthy volunteers and patients with stable cardiovascular disease, why not in patients with myocardial infarction? A key difference

between these cohorts, is that patients with myocardial infarction receive DAPT comprising of aspirin and a $P2Y_{12}$ inhibitor as part of standard of care.[30] In healthy male volunteers, pre-treatment with aspirin did not influence the effect of RIC on platelet aggregation and turnover.[51] However, preclinical studies indicate that $P2Y_{12}$ inhibitors may have direct cardioprotective effects independent of inhibition of platelet-mediated thrombosis. In animal studies, $P2Y_{12}$ inhibitors were shown to reduce infarct size in rabbits, rats and nonhuman primates.[52–55] Furthermore, although $P2Y_{12}$ inhibitors proposed to act on cardiomyocytes and upregulate cardioprotective signaling in a manner analogous to IPC,[56] these drugs failed to reduce infarct size in buffer-perfused hearts, indicating that blood, and specifically platelets, are required to confer cardioprotection.[54,57] There are however some data supporting the concept that clopidogrel may reduce infarct size through the attenuation of reperfusion injury and the protective effect appeared to add to the benefit afforded by ischaemic postconditioning .[55,58] It is therefore possible that the benefits of RIC in STEMI may be attenuated by $P2Y_{12}$ inhibitor treatment[59,60] and one can postulate that RIC may confer greater cardioprotection in patients with persistent high on-treatment platelet reactivity.

The lack of effect of RIC on markers of coagulation in TEG are not altogether surprising. Although RIC in patients with subarachnoid haemorrhage appeared to prolong the prothrombin time and international normalised ratio after at least 4 sessions, values remained within normal range.[56]

We did not observe an effect of RIC on *in vitro* endogenous fibrinolysis. In patients with STEMI, pre-infarction angina (thought to provide IPC) was associated with a significant reduction in the time to achieve thrombolysis-induced reperfusion.[61] This was confirmed in animal studies where recombinant tissue-type plasminogen activator -induced thrombolysis was significantly shortened in animals that received brief antecedent IPC.[62] Our findings of a lack of effect of RIC on fibrinolysis is supported by a study in healthy subjects, where IRI

was shown to induce fibrinolytic dysfunction evidenced by reduced tissue plasminogen activator release that could not be prevented by local IPC or RIC.[63] However, global tests of fibrinolysis, such as performed here, and which give better assessment of global fibrinolytic status than factorial measures such as tissue-plasminogen activator and plasminogen activator inhibitor-1 levels,[20] have not been studies in either animal or human studies.

Limitations

An important limitation of our study is the small sample size. Any observed differences over time or between groups could be due to the play of chance. Furthermore, the exact timeline of effect of RIC on thrombotic status is difficult to conclude, due to the paucity of sampling times. Although a weakness of our study is that mechanistically, we cannot elucidate the cause of the reduced platelet reactivity in patients with RIC, a strength of our work is that we used tests of global thrombotic status, assessing whole blood and in particular, non-anticoagulated blood at high-shear, akin to that in a stenosed coronary vessel, making the findings *in vitro* much more physiologically-relevant, than tests on anticoagulated blood at low shear. With respect to the timing of RIC, a recent meta-analysis showed that RIC protocols that are conducted predominantly before the initiation of reperfusion as opposed to protocols with frequent RIC cycles conducted after reperfusion, conferred more cardioprotection.[64] Although in the ERIC-PPCI study, the start of RIC was before reperfusion, the whole protocol was not always complete before the reperfusion occurred. Upstream start of RIC earlier in the pathway may have improved the outcomes.

Conclusions

Compared to sham treatment, there is a suggestion that RIC may exert a favourable effect on global thrombotic status in patients with STEMI undergoing PPCI, likely through a favourable effect on platelet reactivity. Further research is needed to delineate mechanisms through which RIC may attenuate thrombus formation at high shear stress, and to identify patients who may benefit most from this approach.

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441 REFERENCES

442

443 1. Gorog DA, Lip G. Impaired Spontaneous/Endogenous Fibrinolytic Status as New
444 Cardiovascular Risk Factor? JACC Review Topic of the Week. *J Am Coll Cardiol*
445 2019;**74**:1366–1375.

446

447 2. Hausenloy DJ, Yellon DM. Ischaemic conditioning and reperfusion injury. *Nat Rev*
448 *Cardiol* 2016;**13**:193–209.

449

450 3. Heusch G, Gersh BJ. The pathophysiology of acute myocardial infarction and strategies of
451 protection beyond reperfusion: a continual challenge. *Eur Heart J* 2017; **38**:774-784.

452

453 4. Hausenloy DJ, Botker H, Engstrom T, Erlinge D, Heusch G, Ibanez B, Kloner RA, Ovize
454 M, Yellon DM, Garcia-Dorado D. Targeting reperfusion injury in patients with ST-segment
455 elevation myocardial infarction: trials and tribulations. *Eur Heart J* 2017; **38**:935-941.

456

457 5. Davidson SM, Ferdinandy P, Andreadou I, Bøtker H, Heusch G, Ibáñez B, Ovize M,
458 Schulz R, Yellon DM, Hausenloy DJ, Garcia-Dorado D. Multitarget Strategies to Reduce
459 Myocardial Ischemia/Reperfusion Injury JACC Review Topic of the Week. *J Am Coll*
460 *Cardiol* 2019;**73**:89–99.

461

462 6. Hausenloy DJ, Barrabes JA, Bøtker H, Davidson SM, Lisa F, Downey J, Engstrom T,
463 Ferdinandy P, Carbrera-Fuentes HA, Heusch G, Ibanez B, Iliodromitis EK, Inserte J,
464 Jennings R, Kalia N, Kharbanda R, Lecour S, Marber M, Miura T, Ovize M, Perez-Pinzon
465 MA, Piper H, Przyklenk K, Schmidt M, Redington A, Ruiz-Meana M, Vilahur G, Vinten-

466 Johansen J, Yellon DM, Garcia-Dorado D. Ischaemic conditioning and targeting reperfusion
467 injury: a 30 year voyage of discovery. *Basic Res Cardiol* 2016;**111**:70.
468
469 7. Heusch G, Bøtker H, Przyklenk K, Redington A, Yellon D. Remote Ischemic
470 Conditioning. *J Am Coll Cardiol* 2015;**65**:177–195.
471
472 8. Schmidt M, erup, Konstantinov I, imizu, Li J, Cheung M, White P, Kristiansen S, Sorensen
473 K, Dzavik V, Redington A, Kharbanda R. Intermittent peripheral tissue ischemia during
474 coronary ischemia reduces myocardial infarction through a K ATP -dependent mechanism:
475 first demonstration of remote ischemic perconditioning. *Am J Physiol-Heart* Circ
476 2007;**292**:H1883–H1890.
477
478 9. Hausenloy DJ, Yellon DM. Remote ischaemic preconditioning: underlying mechanisms
479 and clinical application. *Cardiovasc Res* 2008;**79**:377–386.
480
481 10. Thielmann M, Kottenberg E, Kleinbongard P, Wendt D, Gedik N, Pasa S, Price V,
482 Tsagakis K, Neuhäuser M, Peters J, Jakob H, Heusch G. Cardioprotective and prognostic
483 effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass
484 surgery: a single-centre randomised, double-blind, controlled trial. *Lancet* 2013;**382**:597–604.
485
486 11. Meybohm P, Bein B, Brosteanu O, Cremer J, Gruenewald M, Stoppe C, Coburn M,
487 Schaelte G, Böning A, Niemann B, Roesner J, Kletzin F, Strouhal U, Reyher C, Laufenberg-
488 Feldmann R, Ferner M, Brandes IF, Bauer M, Stehr SN, Kortgen A, Wittmann M,
489 Baumgarten G, Meyer-Treschan T, Kienbaum P, Heringlake M, Schön J, Sander M,
490 Treskatsch S, Smul T, Wolwender E, et al. A Multicenter Trial of Remote Ischemic

Preconditioning for Heart Surgery. *New Engl J Medicine* 2015;**373**:1397–1407.

12. Hausenloy DJ, Candilio L, Evans R, Ariti C, Jenkins DP, Kolvekar S, Knight R, Kunst G, Laing C, Nicholas J, Pepper J, Robertson S, Xenou M, Clayton T, Yellon DM, Investigators E. Remote Ischemic Preconditioning and Outcomes of Cardiac Surgery. *New Engl J Medicine* 2015;**373**:1408–1417.

13. Munk K, Andersen N, Schmidt M, Nielsen S, Terkelsen C, Sloth E, Bøtker H, Nielsen T, Poulsen S. Remote Ischemic Conditioning in Patients With Myocardial Infarction Treated With Primary Angioplasty. *Circulation Cardiovasc Imaging* 2010;**3**:656–662.

14. Bøtker H, Kharbanda R, Schmidt MR, Bøttcher M, Kaltoft AK, Terkelsen CJ, Munk K, Andersen NH, Hansen TM, Trautner S, Lassen J, Christiansen E, Krusell LR, Kristensen SD, Thuesen L, Nielsen SS, Rehling M, Sørensen H, Redington AN, Nielsen TT. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet* 2010;**375**:727–734.

15. Lim S, Hausenloy D. Remote Ischemic Conditioning: From Bench to Bedside. *Front Physiol* 2012;**3**:27.

16. Kleinbongard P, Skyschally A, Heusch G. Cardioprotection by remote ischemic conditioning and its signal transduction. *Pflugers Archiv European J Physiology* 2016;**469**:159–181.

17. LINDEN M, WHITTAKER P, FRELINGER A, RNARD M, MICHELSON A, PRZYKLENK K. Preconditioning ischemia attenuates molecular indices of platelet activation-aggregation. *J Thromb Haemost* 2006;**4**:2670–2677.

18. Battipaglia I, Scalone G, Milo M, Franco A, Lanza GA, Crea F. Upper arm intermittent ischaemia reduces exercise-related increase of platelet reactivity in patients with obstructive coronary artery disease. *Heart* 2011;**97**:1298.

19. Stazi A, Scalone G, Laurito M, Milo M, Pelargonio G, Narducci M, Parrinello R, Figliozzi S, Bencardino G, Perna F, Lanza GA, Crea F. Effect of Remote Ischemic Preconditioning on Platelet Activation and Reactivity Induced by Ablation for Atrial Fibrillation. *Circulation* 2014;**129**:11–17.

20. Okafor ON, Gorog DA. Endogenous Fibrinolysis An Important Mediator of Thrombus Formation and Cardiovascular Risk. *Journal of the American College of Cardiology* 2015;**65**:1683–1699.

21. Schanze N, Bode C, Duerschmied D. Platelet Contributions to Myocardial Ischemia/Reperfusion Injury. *Front Immunol* 2019;**10**:1260.

22. Hausenloy DJ, Kharbanda RK, Møller U, Ramlall M, Aarøe J, Butler R, Bulluck H, Clayton T, Dana A, Dodd M, Engstrom T, Evans R, Lassen J, Christensen E, Garcia-Ruiz J, Gorog DA, Hjort J, Houghton RF, Ibanez B, Knight R, Lippert FK, Lønborg JT, Maeng M, Milasinovic D, More R, Nicholas JM, Jensen L, Perkins A, Radovanovic N, Rakhit RD, et al. Effect of remote ischaemic conditioning on clinical outcomes in patients with acute

myocardial infarction (CONDI-2/ERIC-PPCI): a single-blind randomised controlled trial. *Lancet* 2019; **394**:1415-1424

23. Hausenloy DJ, Kharbanda R, Schmidt M, Møller U, Ravkilde J, Jensen L, Engstrøm T, Ruiz J, Radovanovic N, Christensen EF, Sørensen H, Ramlall M, Bulluck H, Evans R, Nicholas J, Knight R, Clayton T, Yellon DM, Bøtker H. Effect of remote ischaemic conditioning on clinical outcomes in patients presenting with an ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Eur Heart J* 2015;**36**:1846–1848.

24. Sharma S, Farrington K, Kozarski R, Christopoulos C, Niespialowska-Steuden M, Moffat D, Gorog DA. Impaired thrombolysis: a novel cardiovascular risk factor in end-stage renal disease. *Eur Heart J* 2013;**34**:354–363.

25. Farag M, Spinthakis N, Gue YX, Srinivasan M, Sullivan K, Wellsted D, Gorog DA. Impaired endogenous fibrinolysis in ST-segment elevation myocardial infarction patients undergoing primary percutaneous coronary intervention is a predictor of recurrent cardiovascular events: the RISK PPCI study. *Eur Heart J* 2019;**40**:295–305.

26. Whiting D, DiNardo JA. TEG and ROTEM: Technology and clinical applications. *Am J Hematol* 2014;**89**:228–232.

27. Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. *Stat Methods Med Res* 2016;**25**:1057–1073.

28. Moses MA, Addison PD, Neligan PC, Ashrafpour H, Huang N, McAllister SE, Lipa JE, Forrest CR, Pang CY. Inducing late phase of infarct protection in skeletal muscle by remote preconditioning: efficacy and mechanism. *Am J Physiology-Regulatory Integr Comp Physiology* 2005;**289**:R1609–R1617.

29. Chapman MJ, Nguyen NQ, Fraser RJ. Gastrointestinal motility and prokinetics in the critically ill. *Current Opinion in Critical Care* 2007;**13**:187–194.

30. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio A, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimský P, ESC. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *European Heart Journal* 2018;**39**:119–177.

31. Marcucci R, Patti G, Calabrò P, Gori A, Grossi G, Cirillo P, Pengo V, Gresele P, Pignatelli P, Antonucci E, Mario C di, Valente S, Palareti G. Antiplatelet treatment in acute coronary syndrome patients: Real-world data from the START-Antiplatelet Italian Registry. *Plos One* 2019;**14**:e0219676.

32. Patel A, Vidula M, Kishore SP, Vedanthan R, Huffman MD. Building the Case for Clopidogrel as a World Health Organization Essential Medicine. *Circulation Cardiovasc Qual Outcomes* 2015;**8**:447–451.

591

592 33. Gorog GD and. Platelet Inhibition in Acute Coronary Syndrome and Percutaneous
593 Coronary Intervention: Insights from the Past and Present. *Thrombosis and Haemostasis*
594 2020;1–14.

595

596 34. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, mcott C,
597 Pakyz R, Tantry US, Gibson Q, Pollin TI, Post W, Parsa A, Mitchell BD, Faraday N, Herzog
598 W, Gurbel PA. Association of cytochrome P450 2C19 genotype with the antiplatelet effect
599 and clinical efficacy of clopidogrel therapy. *JAMA* 2009;**302**:849–857.

600

601 35. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman
602 EM, Macias W, Braunwald E, Sabatine MS. Cytochrome p-450 polymorphisms and response
603 to clopidogrel. *New Engl J Med* 2009;**360**:354–362.

604

605 36. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N, Steg P,
606 Ferrières J, Danchin N, Becquemont L, Investigators F of and. Genetic determinants of
607 response to clopidogrel and cardiovascular events. *New Engl J Med* 2009;**360**:363–375.

608

609 37. Mega JL, Simon T, Collet J-PP, Anderson JL, Antman EM, Bliden K, Cannon CP,
610 Danchin N, Giusti B, Gurbel P, Horne BD, Hulot J-SS, Kastrati A, Montalescot G, Neumann
611 F-JJ, Shen L, Sibbing D, Steg P, Trenk D, Wiviott SD, Sabatine MS. Reduced-function
612 CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with
613 clopidogrel predominantly for PCI: a meta-analysis. *JAMA* 2010;**304**:1821–1830.

614

615 38. Sorich M, Rowland A, McKinnon RA, Wiese MD. CYP2C19 genotype has a greater

effect on adverse cardiovascular outcomes following PCI and in Asian populations treated with clopidogrel: a meta-analysis. *Circulation: Cardiovascular Genetics* 2014;**7**:895-902.

39. Langford E, Wainwright R, Martin J. Platelet Activation in Acute Myocardial Infarction and Unstable Angina Is Inhibited by Nitric Oxide Donors. *Arteriosclerosis Thrombosis Vasc Biology* 1996;**16**:51–55.

40. Trip MD, Cats V, Capelle F van, Vreeken J. Platelet Hyperreactivity and Prognosis in Survivors of Myocardial Infarction. *New Engl J Medicine* 1990;**322**:1549–1554.

41. Gawaz M. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. *Cardiovasc Res* 2004;**61**:498–511.

42. Xu Y, Huo Y, Toufektsian M-C, Ramos SI, Ma Y, Tejani AD, French BA, Yang Z. Activated platelets contribute importantly to myocardial reperfusion injury. *Am J Physiol-heart C* 2006;**290**:H692–H699.

43. Mirabet M, Garcia-Dorado D, Inserte J, Barrabés JA, Lidón R-M, Soriano B, Azevedo M, Padilla F, Agulló L, Ruiz-Meana M, Massaguer A, Pizcueta P, Soler-Soler J. Platelets activated by transient coronary occlusion exacerbate ischemia-reperfusion injury in rat hearts. *Am J Physiol-Heart C* 2002;**283**:H1134–H1141.

44. RØPCKE D, HJORTDAL V, TOFT G, JENSEN M, KRISTENSEN S. Remote ischemic preconditioning reduces thrombus formation in the rat. *J Thromb Haemost* 2012;**10**:2405–2406.

641

642 45. Shan L, Li J, Zu L, Niu C, Ferro A, Zhang Y, Zheng L, Ji Y. Platelet-Derived
643 Microparticles are Implicated in Remote Ischemia Conditioning in a Rat Model of Cerebral
644 Infarction. *Cns Neurosci Ther* 2013;**19**:917–925.

645

646 46. Hata K, Whittaker P, Kloner RA, Przyklenk K. Brief Antecedent Ischemia Attenuates
647 Platelet-Mediated Thrombosis in Damaged and Stenotic Canine Coronary Arteries.
648 *Circulation* 1998;**97**:692–702.

649

650 47. PEDERSEN C, CRUDEN N, SCH M, LAU C, BØTKER H, KHARNDA R, NEWBY D.
651 Remote ischemic preconditioning prevents systemic platelet activation associated with
652 ischemia–reperfusion injury in humans. *J Thromb Haemost* 2011;**9**:404–407.

653

654 48. Kharbanda RK, Peters M, Walton B, Kattenhorn M, Mullen M, Klein N, Vallance P,
655 Deanfield J, MacAllister R. Ischemic Preconditioning Prevents Endothelial Injury and
656 Systemic Neutrophil Activation During Ischemia-Reperfusion in Humans In Vivo.
657 *Circulation* 2001;**103**:1624–1630.

658

659 49. Pasupathy S, Naseem K, Homer-Vanniasinkam S. Effects of warm-up on exercise
660 capacity, platelet activation and platelet–leucocyte aggregation in patients with claudication.
661 *Brit J Surg* 2005;**92**:50–55.

662

663 50. Lanza G, Stazi A, Villano A, Torrini F, Milo M, Laurito M, Flego D, Aurigemma C,
664 Liuzzo G, Crea F. Effect of Remote Ischemic Preconditioning on Platelet Activation Induced
665 by Coronary Procedures. *Am J Cardiol* 2016;**117**:359–365.

51. Rise N, Kristiansen J, Hvas A-M, Grove EL, Würtz M, Neergaard-Petersen S, Kristensen S. Effect of remote ischaemic conditioning on platelet aggregation and platelet turnover. *J Thromb Thrombolys* 2018;**46**:528–533.

52. Yang X-M, Liu Y, Cui L, Yang X, Liu Y, Tandon N, Kambayashi J, Downey JM, Cohen MV. Platelet P2Y12 Blockers Confer Direct Postconditioning-Like Protection in Reperfused Rabbit Hearts. *J Cardiovasc Pharm T* 2013;**18**:251–262.

53. Yang X-M, Liu Y, Cui L, Yang X, Liu Y, Tandon N, Kambayashi J, Downey JM, Cohen MV. Two Classes of Anti-Platelet Drugs Reduce Anatomical Infarct Size in Monkey Hearts. *Cardiovasc Drug Ther* 2013;**27**:109–115.

54. Bell R, Sivaraman V, Kunuthur S, Cohen M, Downey J, Yellon D. Cardioprotective Properties of the Platelet P2Y12 Receptor Inhibitor, Cangrelor: Protective in Diabetics and Reliant Upon the Presence of Blood. *Cardiovasc Drug Ther* 2015;**29**:415–418.

55. Kleinbongard P, Bøtker H, Ovize M, Hausenloy DJ, Heusch G. Co-morbidities and co-medications as confounders of cardioprotection—Does it matter in the clinical setting? *Brit J Pharmacol* 2020; https://doi.org/10.1111/bph.14839,

56. Mayor F, Bilgin-Freiert A, Connolly M, Katsnelson M, Dusick JR, Vespa P, Koch S, Gonzalez NR. Effects of Remote Ischemic Preconditioning on the Coagulation Profile of Patients With Aneurysmal Subarachnoid Hemorrhage: A Case-Control Study. *Neurosurgery* 2013;**73**:808–815.

691

692 57. Cohen MV, Yang X-M, White J, Yellon DM, Bell RM, Downey JM. Cangrelor-Mediated
693 Cardioprotection Requires Platelets and Sphingosine Phosphorylation. *Cardiovasc Drug Ther*
694 2016;**30**:229–232.

695

696 58. Roubille F, Lairez O, Mewton N, Rioufol G, Ranc S, Sanchez I, Cung T, Elbaz M, Piot C,
697 Ovize M. Cardioprotection by clopidogrel in acute ST-elevated myocardial infarction
698 patients: a retrospective analysis. *Basic Res Cardiol* 2012;**107**:275.

699

700 59. Cohen MV, Downey JM. Combined Cardioprotectant and Antithrombotic Actions of
701 Platelet P2Y12 Receptor Antagonists in Acute Coronary Syndrome. *J Cardiovasc Pharm T*
702 2013;**19**:179–190.

703

704 60. Przyklenk K, Whittaker P. Ischemic Conditioning Attenuates Platelet-Mediated
705 Thrombosis. *J Cardiovasc Pharm T* 2017;**22**:391–396.

706

707 61. Andreotti F, Pasceri V, Hackett DR, Davies GJ, Haider AW, Maseri A. Preinfarction
708 Angina as a Predictor of More Rapid Coronary Thrombolysis in Patients with Acute
709 Myocardial Infarction. *New Engl J Med* 1996;**334**:7–12.

710

711 62. Przyklenk K, Whittaker P. Brief Antecedent Ischemia Enhances Recombinant Tissue
712 Plasminogen Activator–Induced Coronary Thrombolysis by Adenosine-Mediated
713 Mechanism. *Circulation* 2000;**102**:88–95.

714

715 63. Pedersen CM, Barnes G, Schmidt MR, Bøtker H, Kharbanda RK, Newby DE, Cruden

NL. Ischaemia–reperfusion injury impairs tissue plasminogen activator release in man. *Eur Heart J* 2012;**33**:1920–1927.

64. Haller PM, Vargas KG, Haller MC, Piackova E, Wojta J, Gyöngyösi M, Gersh BJ, Kiss A, Podesser BK, Huber K. Remote ischaemic conditioning for myocardial infarction or elective PCI: systematic review and meta-analyses of randomised trials. *European Hear J Acute Cardiovasc Care* 2018;2048872618784150.

Figure legends

Figure 1. ERIC-PPCI study flowchart

Flowchart in black represents the ERIC-PPCI main study, whereas in blue represents the thrombosis substudy. Blood samples were taken at three time points, 1) baseline upon arrival to the catheterisation laboratory and at randomisation 2) at clinical stabilisation, just prior to hospital discharge, and 3) at 6-8 weeks follow-up.

PIS: patient information sheet, SAEs: serious adverse events, NSAEs: non-serious adverse events

Figure 2. Distribution of OT and LT at the pre-specified time points

OT= occlusion time, LT= lysis time. *P<0.01 compared to baseline. OT at baseline vs. discharge (paired t-test: mean difference 92s, [95%CI 66.61-117.57], p<0.001). OT at baseline vs. 30 days (Mann-Whitney U test: mean difference 193s, [95%CI 158.29-229.61], p<0.001).

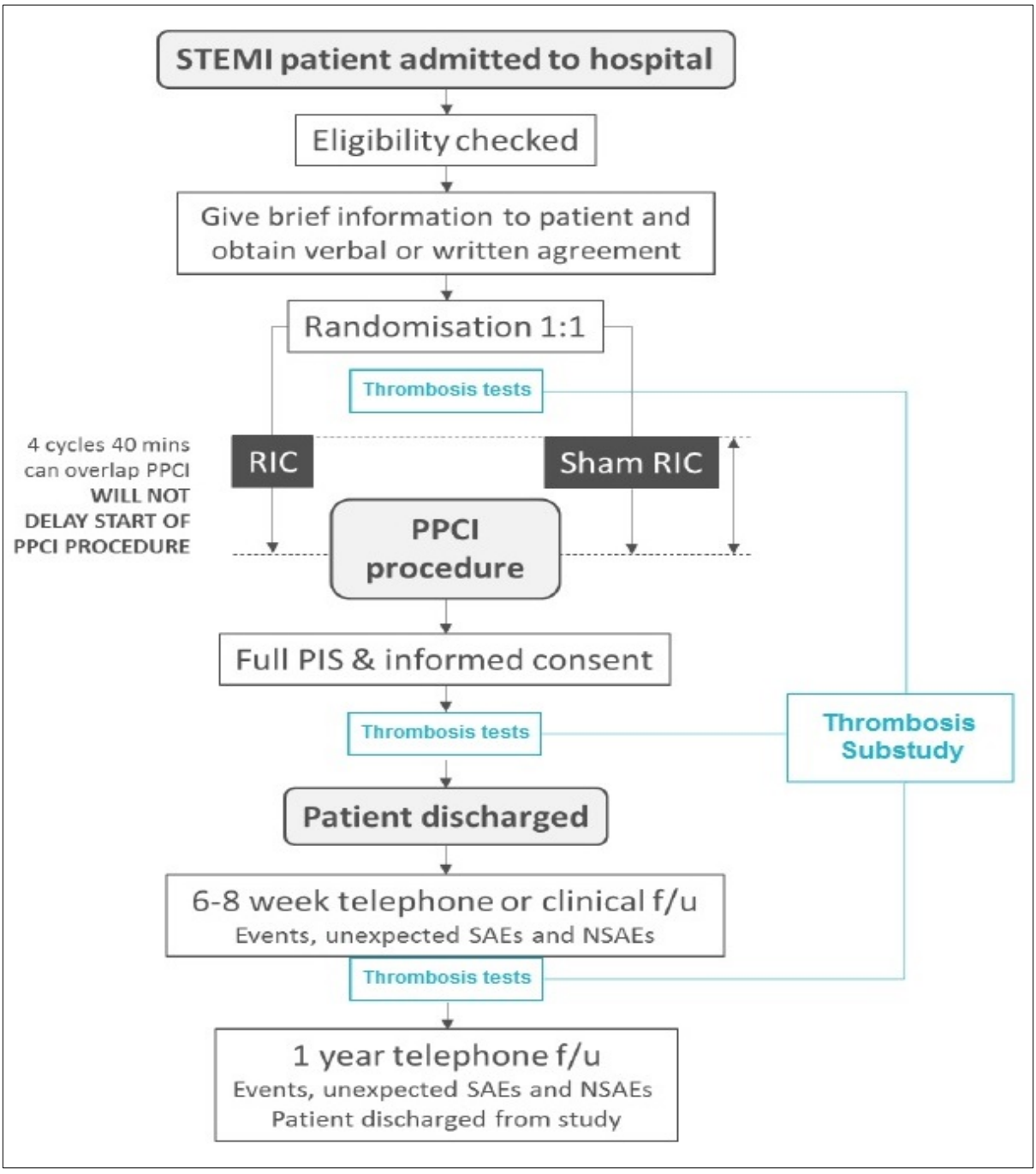
Figure 3. Distribution of OT at the pre-specified time points between the study arms

Occlusion time (OT) was significantly prolonged at hospital discharge in RIC group compared to sham RIC group. * Comparison between RIC and sham, P <0.05. † difference within group compared to baseline P<0.001. Comparison made using ANCOVA.

Figure 4. Distribution of LT at the pre-specified time points between the study arms

There was no significant difference in lysis time (LT) between the two study arms at any time point. Comparison made using ANCOVA.

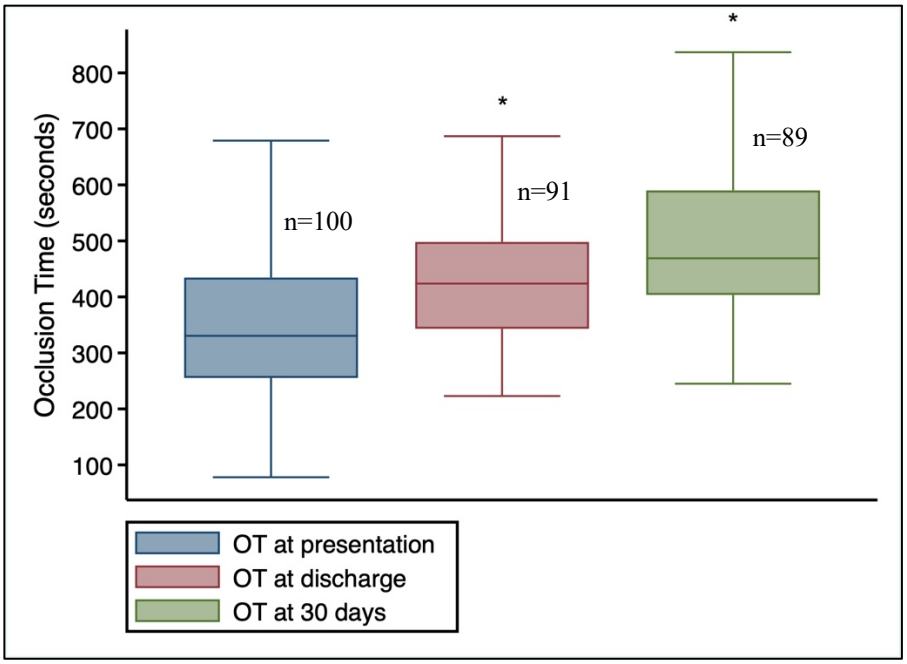
747 **Figure 1.**



748

Figure 2.

A


Occlusion Time (seconds)
800
700
600
500
400
300
200
100
n=100
n=91
n=89
*
*
OT at presentation
OT at discharge
OT at 30 days

B

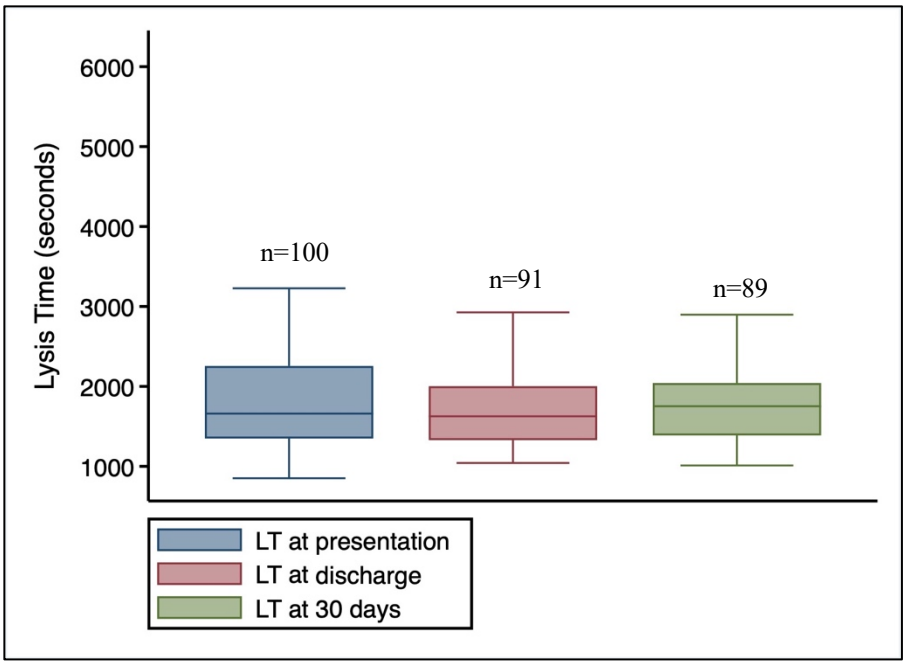

Lysis Time (seconds)
6000
5000
4000
3000
2000
1000
n=100
n=91
n=89
LT at presentation
LT at discharge
LT at 30 days

Figure 3.

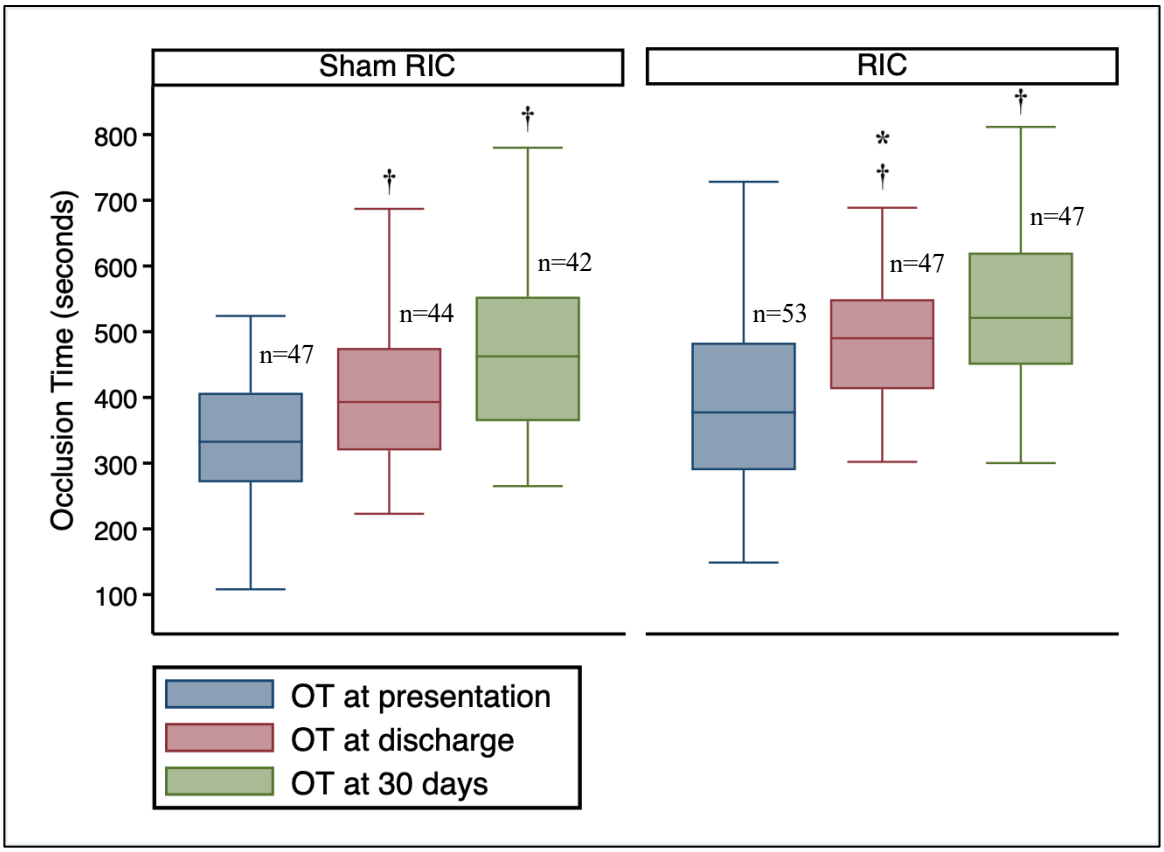
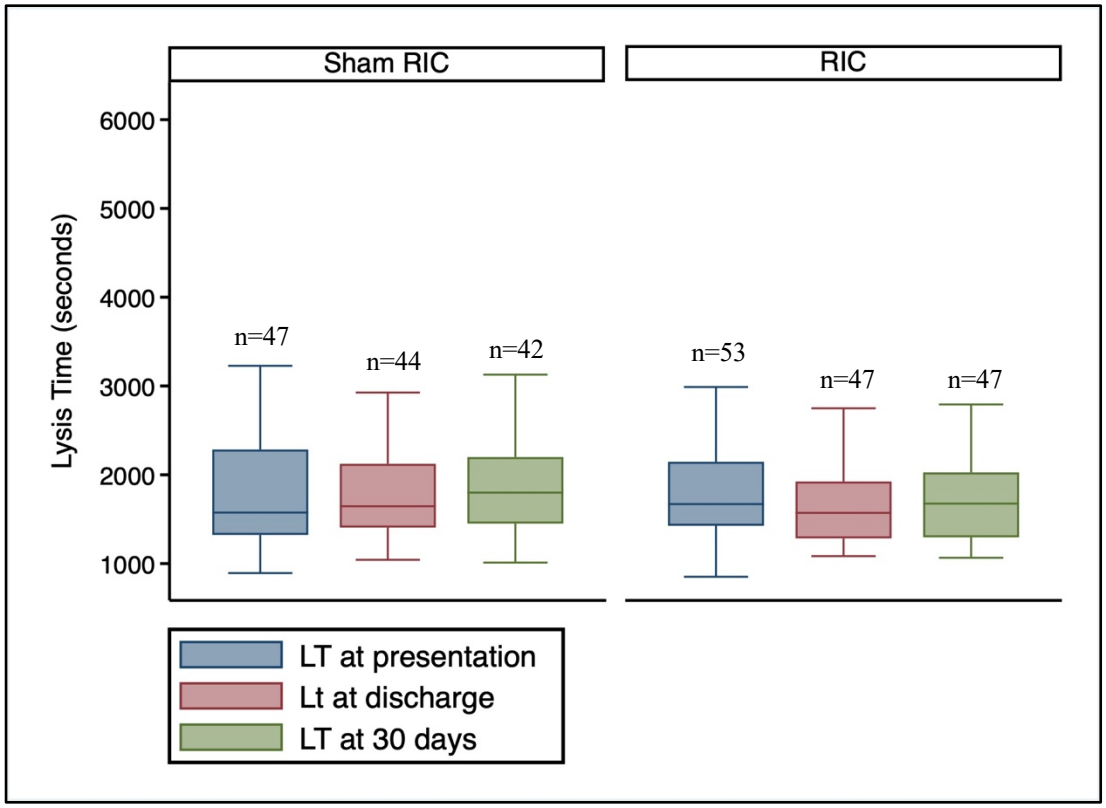

Sham RIC
RIC
Occlusion Time (seconds)
800
700
600
500
400
300
200
100
n=47
n=44
n=42
n=53
n=47
n=47
†
*
OT at presentation
OT at discharge
OT at 30 days

Figure 4.


Sham RIC
RIC
Lysis Time (seconds)
6000
5000
4000
3000
2000
1000
n=47
n=44
n=42
n=53
n=47
n=47
LT at presentation
Lt at discharge
LT at 30 days

760 **Table 1. TEG indices and definitions**

Reaction Time (R) [min]	Measures the time from the start of a sample run until the first significant level of detectable clot formation. R is shortened by hypercoagulable conditions
Kinetics (K) [min]	Measures the time from R until a fixed level of clot strength is reached. K is shortened by hypercoagulable conditions. When MA <20 mm, K is undefined
Angle [degrees]	Represents the rate of clot formation and reflects fibrinogen activity. Angle relates to K, since both are a function of the rate of clot formation. Angle is larger by hypercoagulable conditions
Maximum Amplitude (MA) [mm]	Represents whole clot strength and reflects many aspects of clot formation including platelet number and function as well as the fibrin contribution to clot strength. MA is larger by hypercoagulable conditions
LY30 [%]	Represents the percentage of clot which has lysed after 30 minutes of MA
LY60 [%]	Represents the percentage of clot which has lysed after 60 minutes of MA
Time to Maximum Amplitude (TMA) [min]	Measures the time to form maximum clot strength

761

762 **Table 2. Baseline Patient Characteristics**

	Whole Group (n=100)	Sham RIC (n=47)	RIC (n=53)	P Value
Age, yrs	65.2±13.6	65.1±13.1	65.4±14.1	0.903
Male	79 (79.0)	37 (78.7)	42 (79.2)	1.000
Caucasian	93 (93.0)	46 (97.9)	47 (88.7)	0.117
BMI	26.7±4.2	26.9±4.8	26.6±3.6	0.673
TIMI score	3.1±2.4	2.9±2.3	3.3±2.5	0.467
Diabetes mellitus	20 (20.0)	7 (14.9)	13 (24.5)	0.317
Active smoker	27 (27.0)	15 (31.9)	12 (22.6)	0.369
Hypertension	44 (44.0)	20 (42.6)	24 (45.3)	0.842
Family history of premature IHD	26 (26.0)	13 (27.7)	13 (24.5)	0.820
Prior MI	9 (9.0)	3 (6.4)	6 (11.3)	0.495
Prior PCI	8 (8.0)	3 (6.4)	5 (9.4)	0.719
Renal insufficiency	4 (4.0)	2 (4.3)	2 (3.8)	1.000
PVD	3 (3.0)	3 (6.4)	0	0.100
Prior CVA	4 (4.0)	1 (2.1)	3 (5.7)	0.620
Prior statin use	26 (26.0)	14 (29.8)	12 (22.6)	0.496
Prior aspirin use	16 (16.0)	5 (10.6)	11 (20.8)	0.186
Prior $P2Y_{12}$ inhibitor use	1 (1.0)	0	1 (1.9)	1.000
Initial $P2Y_{12}$ inhibitor loading agent				
Clopidogrel	76 (76.0)	37 (78.7)	39 (73.6)	0.642
Ticagrelor	20 (20.0)	8 (17.0)	12 (22.6)	0.618
Cangrelor	4 (4.0)	2 (4.3)	2 (3.8)	1.000
Morphine prior to blood sample	59 (59.0)	26 (55.3)	33 (62.3)	0.544
Time from $P2Y_{12}$ inhibitor loading to first blood sample (min)	46.9±21.9	46.9±19.1	46.9±24.2	0.979
Medications prior to hospital discharge				
Aspirin	94 (94.0)	45 (95.7)	49 (92.5)	1.000
Clopidogrel	12 (12.0)	7 (14.9)	5 (9.4)	0.540
Ticagrelor	82 (82.0)	38 (80.9)	44 (83.0)	0.800
Beta-blocker	91 (91.0)	44 (93.6)	47 (88.7)	1.000
ACE inhibitor	93 (93.0)	45 (95.7)	48 (90.6)	1.000
Calcium antagonist	6 (6.0)	1 (2.1)	5 (9.4)	0.206
Statin	92 (92.0)	45 (95.7)	47 (88.7)	0.496
Nitrate	2 (2.0)	0	2 (3.8)	0.497
Insulin	3 (3.0)	2 (4.3)	1 (1.9)	0.599

763

764 Values are mean ± standard deviation or n (%). Renal insufficiency was defined as creatinine levels >177 μmol/L.
765 Prior statin, aspirin or $P2Y_{12}$ inhibitor use defined as regular statin, aspirin or $P2Y_{12}$ inhibitor use before
766 hospitalisation. Family history of premature IHD was defined as a diagnosis of IHD in a first-degree relative under
767 the age of 60.
768 ACE: angiotensin-converting enzyme, BMI: body mass index, CVA: cerebrovascular accident, IHD: ischaemic
769 heart disease, MI: myocardial infarction, PCI: percutaneous coronary intervention, PVD: peripheral vascular
770 disease, TIMI: Thrombolysis in Myocardial Infarction.

771 **Table 3. Haematological and biochemical profiles**

	Whole Group (n=100)	Sham RIC (n=47)	RIC (n=53)	P Value
Haemoglobin (g/L)	138±19	136±19	139±19	0.400
Haematocrit (%)	41±6	40±6	41±5	0.516
Neutrophil count (x10^9/L)	8.6±2.9	8.6±2.8	8.6±3.1	0.938
Platelet count (x10^9/L)	259±77	258±78	260±77	0.923
Serum albumin (g/L) *	43±3.7	42±3.8	43±3.7	0.243
Sodium (mmol/L)	138±3	138±2	138±3	0.789
Creatinine (µmol/L)	91±37	94±49	89±23	0.513
Peak troponin T (ng/L) *	2223 [1072-3796]	2014 [993-3606]	2301 [1074-3945]	0.474
Fibrinogen (g/L)	4.6±1.3	4.6±1.1	4.7±1.5	0.605
PT (sec)	11.8±1.1	11.8±1.0	11.9±1.2	0.728
aPTT (sec)	28.1±3.6	27.5±3.4	28.6±3.7	0.175
Total cholesterol (mmol/L)	5.1±1.2	4.9±1.2	5.3±1.1	0.121
LDL cholesterol (mmol/L)	2.9±0.8	2.9±0.9	2.9±0.7	0.867
Hs C-reactive protein (mg/l) *	3 [1-8]	3 [2-8]	2 [1-8]	0.273

772

773 Values are mean ± standard deviation, except * where values are median [IQR]. aPTT: activated partial
774 thromboplastin time; LDL: low-density lipoprotein; PT: prothrombin time. All values measured at presentation,
775 except peak troponin T.
776 Normal values: haemoglobin 130-180 g/L (males) and 115-165 g/L (females); haematocrit 40-52% (males) and
777 36-47% (females); neutrophil count 2-7.5 x10^9/L; platelet count 150-400 x10^9/L; serum albumin 34-54 g/L; serum
778 sodium 135-145 mmol/L, creatinine 60-110 µmol/L (males) and 45-90 µmol/L (females); troponin T <14 ng/L
779 (Elecsys high-sensitivity assay, Roche Diagnostics); fibrinogen 2–4 g/L; PT 11-13.5 seconds; aPTT 25-35
780 seconds; total cholesterol ≤4.0 mmol/L; LDL cholesterol ≤2.0 mmol/L; high sensitivity C-reactive protein 0–3
781 mg/l.
782

783 **Table 4. Angiographic, Interventional and Echocardiographic Patient Characteristics**

	Whole Group (n=100)	Sham RIC (n=47)	RIC (n=53)	P Value
Complete (>70%) ST-segment resolution on ECG pre-PPCI	9 (9.0)	5 (10.6)	4 (7.5)	0.731
Systolic blood pressure (mmHg) on arrival *	130±24	133±26	128±23	0.338
Diastolic blood pressure (mmHg) on arrival *	78±16	80±16	76±15	0.275
Heart rate (bpm) on arrival *	79±18	78±18	80±19	0.752
Killip classification score >2	4 (4.0)	2 (4.3)	2 (3.8)	1.000
Radial access	93 (93.0)	42 (89.4)	51 (96.2)	0.249
1-vessel disease	54 (54.0)	23 (48.9)	31 (58.5)	0.422
2-vessel disease	31 (31.0)	17 (36.2)	14 (26.4)	0.387
3-vessel disease	15 (15.0)	7 (14.9)	8 (15.1)	1.000
Culprit vessel LAD	44 (44.0)	16 (34.0)	27 (50.9)	0.107
GPI (Tirofiban) use	32 (32.0)	16 (34.0)	16 (30.2)	0.830
Thrombus aspiration	7 (7.0)	3 (6.4)	4 (7.5)	1.000
DES implantation	95 (95.0)	43 (91.5)	52 (98.1)	0.184
Stent diameter <3 mm	31 (31.0)	16 (34.0)	15 (28.3)	0.388
TIMI 2/3 angiographic flow pre-PPCI	23 (23.0)	10 (21.3)	13 (24.5)	0.813
TIMI 2/3 angiographic flow post-PPCI	99 (99.0)	47 (100)	52 (98.1)	1.000
Myocardial blush grade 2/3 post-PPCI	95 (95.0)	46 (97.9)	49 (92.5)	1.000
Door to first device time, min	29 [23-36]	29 [21-33]	30 [24-53]	0.179
Call to first device time, min	101 [76-134]	98 [76-131]	103 [75-136]	0.882
Pain to first device time, min	162 [118-263]	170 [119-276]	155 [117-235]	0.519
Left ventricular function				
Normal (EF ≥55%)	34 (34.0)	16 (34.0)	18 (33.9)	1.000
Mildly impaired (EF 45–54%)	36 (36.0)	16 (34.0)	20 (37.8)	0.835
Moderately impaired (EF 36–44%)	23 (23.0)	13 (27.7)	10 (18.9)	0.346
Severely impaired (EF ≤35%)	7 (7.0)	2 (4.3)	5 (9.4)	0.442

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785 Values are median [IQR] or n (%), except * where values are mean ± standard deviation. Left ventricular function
786 was assessed by echocardiography prior to hospital discharge.

787 DES: drug eluting stent, EF: ejection fraction, GPI: glycoprotein IIb/IIIa inhibitor, LAD: left anterior descending
788 coronary artery, MI: myocardial infarction, PPCI: primary percutaneous coronary intervention, TIMI:
789 Thrombolysis in Myocardial Infarction.

790 Door to first device time was the time interval between the arrival of a patient at the hospital and the time of first
791 intracoronary device use (defined as time of first balloon or stent inflation; or use of thrombectomy or angioplasty
792 wire if these re-established flow). Call to device time was the time interval between the first call for help and first

device time. Pain to device time was the time interval between the onset of symptoms and the first intracoronary device use.

795 **Table 5. Tests of thrombotic status**

	Whole Group (n=100)	Sham RIC (n=47)	RIC (n=53)	P value
Global Thrombosis Test (GTT)				
Baseline				
OT [sec] *	337±129	329±98	349±151	0.444
LT [sec]	1660[1348-2255]	1574[1323-2284]	1670[1426-2146]	0.777
At discharge				
OT [sec] *	430±107	403±105	454±105	0.025
LT [sec]	1626[1328-2002]	1646[1406-2123]	1571[1284-1924]	0.241
At 6-8 weeks				
OT [sec] *	493±132	471±132	512±130	0.144
LT [sec]	1752[1387-2042]	1799[1451-2199]	1675[1296-2026]	0.227
Thromboelastography (TEG)				
Baseline (native blood sample)				
Reaction Time (R) [min]	8.2[5.9-9.5]	8.2[5.9-9.6]	8.2[6.1-9.3]	0.841
Kinetics (K) [min]	2.5[1.9-3.8]	2.2[1.8-3.4]	2.7[2.1-3.9]	0.124
Angle [degrees]	56[45-64]	59[47-66]	53[39-62]	0.204
Maximum Amplitude (MA) [mm]	73[67-78]	72[69-78]	73[66-78]	0.889
LY30 [%]	0.2[0-1.6]	0.7[0-3.5]	0.1[0-1.1]	0.099
LY60 [%]	2.8[0.9-5.1]	3.5[1.2-7.2]	2.5[0.6-4.5]	0.279
Time to Maximum Amplitude (TMA) [min]	28.2[24.2-34.8]	26.2[23.4-32.9]	30.5[24.8-36.9]	0.242
At discharge (native blood sample)				
Reaction Time (R) [min]	9.1[6.3-11.8]	10.6[6.3-11.8]	8.9[6.5-11.4]	0.865
Kinetics (K) [min]	3.2[1.9-4.0]	3.5[1.9-3.9]	2.7[1.9-4.4]	0.864
Angle [degrees]	53[46-65]	52[49-65]	58[41-64]	0.884
Maximum Amplitude (MA) [mm]	73[68-77]	73[69-77]	72[66-79]	0.990

LY30 [%]	0.8[0-4.7]	0.6[0.1-8.0]	1.1[0-3.9]	0.741
LY60 [%]	3.5[1.2-9.7]	3.5[1.9-13.4]	3.7[1.2-9.5]	0.576
Time to Maximum Amplitude (TMA) [min]	30.6[22.2-33.6]	31.6[22.2-34.3]	27.0[22.2-32.1]	0.444
At 6-8 weeks (native blood sample)				
Reaction Time (R) [min]	9.8[7.6-12.3]	9.7[8.0-12.3]	10.0[7.2-12.3]	0.882
Kinetics (K) [min]	2.6[1.9-3.6]	2.8[1.9-3.5]	2.6[1.9-3.8]	0.974
Angle [degrees]	58[49-65]	58[49-65]	61[49-64]	0.691
Maximum Amplitude (MA) [mm]	75[71-79]	76[69-79]	74[72-79]	0.817
LY30 [%]	1.0[0.1-2.2]	1.2[0.1-2.1]	0.6[0.1-2.9]	0.855
LY60 [%]	4.0[1.6-6.1]	4.0[1.8-6.1]	3.3[1.6-6.3]	0.585
Time to Maximum Amplitude (TMA) [min]	29.1[22.1-34.8]	27.0[20.6-33.7]	29.7[24.9-35.8]	0.260
Baseline (Kaolin added)				
Reaction Time (R) [min]	5.1[3.2-5.9]	5.2[3.2-6.1]	5.0[3.5-5.9]	0.750
Kinetics (K) [min]	1.2[1.1-1.4]	1.2[1.0-1.6]	1.2[1.1-1.4]	0.873
Angle [degrees]	72[67-74]	71[67-75]	72[69-74]	0.811
Maximum Amplitude (MA) [mm]	76[72-81]	76[71-81]	76[74-79]	0.812
LY30 [%]	1.1[0.2-4.3]	1.2[0-3.7]	1.0[0.3-5.4]	0.404
LY60 [%]	4.5[2.0-8.1]	3.6[1.5-7.7]	5.4[2.3-8.2]	0.439
Time to Maximum Amplitude (TMA) [min]	20.8[17.7-23.8]	21.5[18.4-24.4]	19.6[16.9-23.8]	0.300
At discharge (Kaolin added)				
Reaction Time (R) [min]	5.3[3.6-7.2]	5.9[3.8-7.2]	5.2[3.6-7.3]	0.919
Kinetics (K) [min]	1.3[1.1-1.5]	1.3[1.1-1.5]	1.2[1.2-1.5]	0.859
Angle [degrees]	72[67-75]	71[68-75]	72[67-74]	0.841
Maximum Amplitude (MA) [mm]	78[74-81]	76[75-80]	78[74-82]	0.606
LY30 [%]	2.1[0.7-4.9]	1.8[0.6-4.8]	3.1[0.9-5.2]	0.624
LY60 [%]	5.9[3.3-10.5]	5.1[3.2-9.5]	7.3[4.2-12.0]	0.473

Time to Maximum Amplitude (TMA) [min]	20.7[17.9-23.4]	21.1[17.8-23.4]	20.2[18.4-23.2]	0.753
At 6-8 weeks (Kaolin added)				
Reaction Time (R) [min]	5.7[3.9-7.3]	5.0[3.3-7.3]	6.6[4.3-7.3]	0.706
Kinetics (K) [min]	1.4[1.1-1.7]	1.4[1.0-1.6]	1.4[1.2-1.8]	0.490
Angle [degrees]	71[66-74]	71[69-75]	71[66-74]	0.544
Maximum Amplitude (MA) [mm]	78[75-82]	78[77-82]	77[74-82]	0.530
LY30 [%]	2.0[0.5-3.8]	2.1[0.1-3.1]	2.0[0.5-4.4]	0.367
LY60 [%]	5.0[2.4-7.5]	5.3[2.1-7.4]	3.7[2.5-7.5]	0.786
Time to Maximum Amplitude (TMA) [min]	21.0[17.4-25.8]	21.0[17.9-23.7]	20.7[17.4-25.9]	0.858

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797 Values are median [IQR] except * where are mean ± standard deviation.

798 LT: lysis time, OT: occlusion time. For explanation of abbreviation of TEG indices, see
799 Table 2.

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