

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

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

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47 **Abstract**

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

52 **Methods and Results:** In a [pre-specified](#) substudy of the Effect of Remote Ischaemic  
53 Conditioning on clinical outcomes in STEMI patients undergoing Primary Percutaneous  
54 Coronary Intervention (ERIC-PPCI) trial, we assessed the effect of RIC on thrombotic status.  
55 Patients presenting with STEMI were randomised to immediate RIC consisting of an  
56 automated autoRIC™ cuff on the upper arm inflated to 200mmHg for 5 minutes and deflated  
57 for 5 minutes for 4 cycles (n=53) or sham (n=47). Venous blood was tested at presentation,  
58 discharge (48 h) and 6-8 weeks, to assess platelet reactivity, coagulation and endogenous  
59 fibrinolysis using the Global Thrombosis Test and thromboelastography (TEG). Baseline  
60 thrombotic status was similar in the 2 groups. At discharge, there was some evidence that the  
61 time to *in vitro* thrombotic occlusion under high shear stress was longer with RIC compared  
62 to sham (454±105s vs. 403±105s; mean difference 50.1s; 95% confidence interval [CI] 93.7-  
63 6.4, P=0.025), but this was no longer apparent at 6-8 weeks. There was no difference in clot  
64 formation or endogenous fibrinolysis between the study arms at any time-point.

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

69 Word count: [259 words](#)

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71 **Abbreviations**

72 ADP = adenosine diphosphate

73 DAPT = dual antiplatelet medication

74 GTT = Global Thrombosis Test

75 IPC = ischaemic preconditioning

76 IR = ischaemia-reperfusion

77 IRI = ischaemia-reperfusion injury

78 LT = lysis time

79 OT = occlusion time

80 PPCI = primary percutaneous coronary intervention


81 RIC = remote ischaemic preconditioning


82 STEMI = ST-segment elevation myocardial infarction

83 TEG = thromboelastography

84

## 85 **Introduction**

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

97 Ischaemic preconditioning (IPC) refers to the ability of brief, cyclic periods of ischaemia and  
98 reperfusion (IR) to render the myocardium more resistant to a subsequent ischaemic insult. In  
99 animal models, IPC has been shown to reduce infarct size and to enhance recovery of  
100 contractile function of the myocardial region at risk.  Remote ischaemic conditioning (RIC)  
101 involves the application of one or more brief cycles of IR to a “remote” organ (such as the  
102 arm or leg) and in animal models, has been shown to reduce infarct size and IRI.<sup>7-9</sup>

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

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

126

127

128 **Methods**

129 *Study design and population*

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

147

148 *Trial treatment protocol*

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

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

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

162

### 163 *Blood sampling technique*

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

175

176 *Assessment of global thrombotic status*

177 Global Thrombosis Test (GTT)

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

190 Thromboelastography (TEG)

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

198



199 *Study end-points*

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

204 *Data collection and follow-up*

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

208

209 *Statistical analysis*

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

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

223

224

## 225 **Results**

226

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

236

### 237 *Global Thrombosis Test (GTT) results*

238 In the whole cohort (n=100), OT increased from baseline to hospital discharge ( $338\pm 129$ s vs.  
239  $430\pm 107$ s,  $p<0.001$ ) and further increased at 6-8 weeks (baseline vs. 6-8 weeks  $338\pm 129$ s vs.  
240  $493\pm 132$ s,  $p<0.001$ )(Figure 2A).

241 Baseline OT was similar in the RIC and sham groups, with mean difference 19.65s (95%  
242 confidence interval [CI] 69.41-70.36) (Table 5, Figure 3). However, there was some evidence  
243 that OT at hospital discharge was prolonged in RIC group compared to sham ( $454\pm 105$ s vs.  
244  $403\pm 105$ s; mean difference 50.1s; 95% CI 6.4-93.7,  $P = 0.025$ ), but this was less apparent at

245 6-8 weeks follow-up ( $538\pm142$ s vs.  $511\pm142$ s, mean difference 27.5s; 95%CI 102.5- 47.5,  
246  $P=0.818$ ) (Table 5, Figure 3).

247 Distribution of LT at the prespecified time points is shown in Figure 2-B. There was no  
248 evidence for a difference in LT between the two study arms at any of the time points (Figure  
249 4 and Table 5).

250

#### 251 *Thromboelastography (TEG) results*

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

255

## 256 **DISCUSSION**

257

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

266 The encouraging results of this substudy contrast with the neutral results of the main CONDI-  
267 2/ERIC-PPCI trial, in which no difference was seen between the RIC and the control groups  
268 with respect to the combined primary endpoint of cardiac death or hospitalisation for heart

269 failure at 12 months (HR 1.10; 95% CI 0.91–1.32; P = 0.32), demonstrating that RIC, applied  
270 as an adjunct to PPCI, did not improve clinical outcomes in STEMI patients. The discrepancy  
271 between the findings of our small substudy and the main trial may simply be due to the play  
272 of chance in a small sample. However, if these results are real, and RIC results in reduced  
273 platelet reactivity at 48h post-PPCI, it would not be surprising if this in fact had no effect on  
274 outcomes. The reduction in platelet reactivity at 48h may be too late to influence reperfusion  
275 and infarct size, or to favourably impact on any reperfusion injury following PPCI. This  
276 might indicate that earlier application of such RIC may have improved outcomes, although in  
277 the main CONDI-2/ERIC-PPCI trial, there were no differences in clinical outcomes whether  
278 RIC was performed in the ambulance or in hospital. Another consideration is that platelet  
279 reactivity is a strong determinant of ischaemic outcomes, in particular in the highest risk  
280 patients. Although acute stent thrombosis is likely multifactorial in aetiology, it has been  
281 been related in part to enhanced platelet reactivity, and so it is possible that a beneficial effect  
282 in reducing platelet reactivity could reduce the occurrence of acute stent thrombosis, although  
283 there was no signal for this in the main CONDI-2/ERIC-PPCI trial, where the occurrence of  
284 myocardial infarction at 30 days was similar in the RIC and sham arms. The CONDI-  
285 2/ERIC-PPCI trial excluded many patients with anterior STEMI, since these often exhibit left  
286 bundle branch block, and patients with cardiogenic shock who were unable to give consent.  
287 Patients with cardiogenic shock are not only at very high cardiovascular risk with 30-50%  
288 risk of death or recurrent ischaemic events over the subsequent 30 days, but shock can also  
289 limit the effectiveness of orally-administered antithrombotic medications due to delayed drug  
290 administration, reduced gastrointestinal blood flow and motility, delayed gastric emptying  
291 and gastrointestinal absorption<sup>29</sup>- so these patients may have the most to gain from  
292 approaches that reduce platelet reactivity. Since the effect on platelet reactivity was no longer  
293 apparent at 6-8 weeks, this may explain the lack of effect on long term ischaemic outcomes.

294 Whilst current guidelines advocate use of the more potent P2Y<sub>12</sub> inhibitors ticagrelor and  
295 prasugrel in patients with STEMI,<sup>30</sup> this also comes at a greater price of bleeding.  
296 Clopidogrel continues to be used in a significant number of ACS patients in high income  
297 countries,<sup>31</sup> and also for financial reasons in low income countries.<sup>32</sup> Up to a third of ACS  
298 patients demonstrate inadequate platelet inhibition in response to clopidogrel.<sup>33</sup> This is  
299 explained in part by polymorphisms in the gene encoding the hepatic enzyme *CYP2C19*,  
300 which transforms clopidogrel to its active metabolite, that can result in 5-12% variation in  
301 platelet inhibition.<sup>34</sup> There is ethnic variation in the prevalence of the loss-of-function  
302 *CYP2C19* 618G>A\*2 allele, affecting some 30% of Caucasians and 50% of East Asians.<sup>33</sup>  
303 Homozygotes for the *CYP2C19*\*2 and less common *CYP2C19*\*3 LoF alleles are poor  
304 metabolizers, and heterozygotes are intermediate metabolizers of clopidogrel, with high-on  
305 clopidogrel platelet reactivity and increased risk of adverse cardiovascular events, including  
306 AMI and stent thrombosis.<sup>35-37</sup> The association of *CYP2C19* genotype with increased  
307 cardiovascular risk appears greatest in those undergoing PCI, and the risk is greater in Asians  
308 than in whites.<sup>38</sup> Enhancing platelet inhibition with RIC in patients who are receiving  
309 clopidogrel may be particularly advantageous in such patients.

310

### 311 *Possible mechanisms*

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

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

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

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

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

342 between these cohorts, is that patients with myocardial infarction receive DAPT comprising  
343 of aspirin and a P2Y<sub>12</sub> inhibitor as part of standard of care.<sup>30</sup> In healthy male volunteers, pre-  
344 treatment with aspirin did not influence the effect of RIC on platelet aggregation and  
345 turnover.<sup>51</sup> However, preclinical studies indicate that P2Y<sub>12</sub> inhibitors may have direct  
346 cardioprotective effects independent of inhibition of platelet-mediated thrombosis. In animal  
347 studies, P2Y<sub>12</sub> inhibitors were shown to reduce infarct size in rabbits, rats and nonhuman  
348 primates.<sup>52-55</sup> Furthermore, although P2Y<sub>12</sub> inhibitors proposed to act on cardiomyocytes and  
349 upregulate cardioprotective signaling in a manner analogous to IPC,<sup>56</sup> these drugs failed to  
350 reduce infarct size in buffer-perfused hearts, indicating that blood, and specifically platelets,  
351 are required to confer cardioprotection.<sup>54,57</sup> There are however some data supporting the  
352 concept that clopidogrel may reduce infarct size through the attenuation of reperfusion injury  
353 and the protective effect appeared to add to the benefit afforded by ischaemic  
354 postconditioning.<sup>55,58</sup> It is therefore possible that the benefits of RIC in STEMI may be  
355 attenuated by P2Y<sub>12</sub> inhibitor treatment<sup>59,60</sup> and one can postulate that RIC may confer greater  
356 cardioprotection in patients with persistent high on-treatment platelet reactivity.

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

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

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

372

### 373 *Limitations*

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

388

### 389 *Conclusions*



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

395

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723

724 **Figure legends**

725 **Figure 1. ERIC-PPCI study flowchart**

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

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

732

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

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

738

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

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

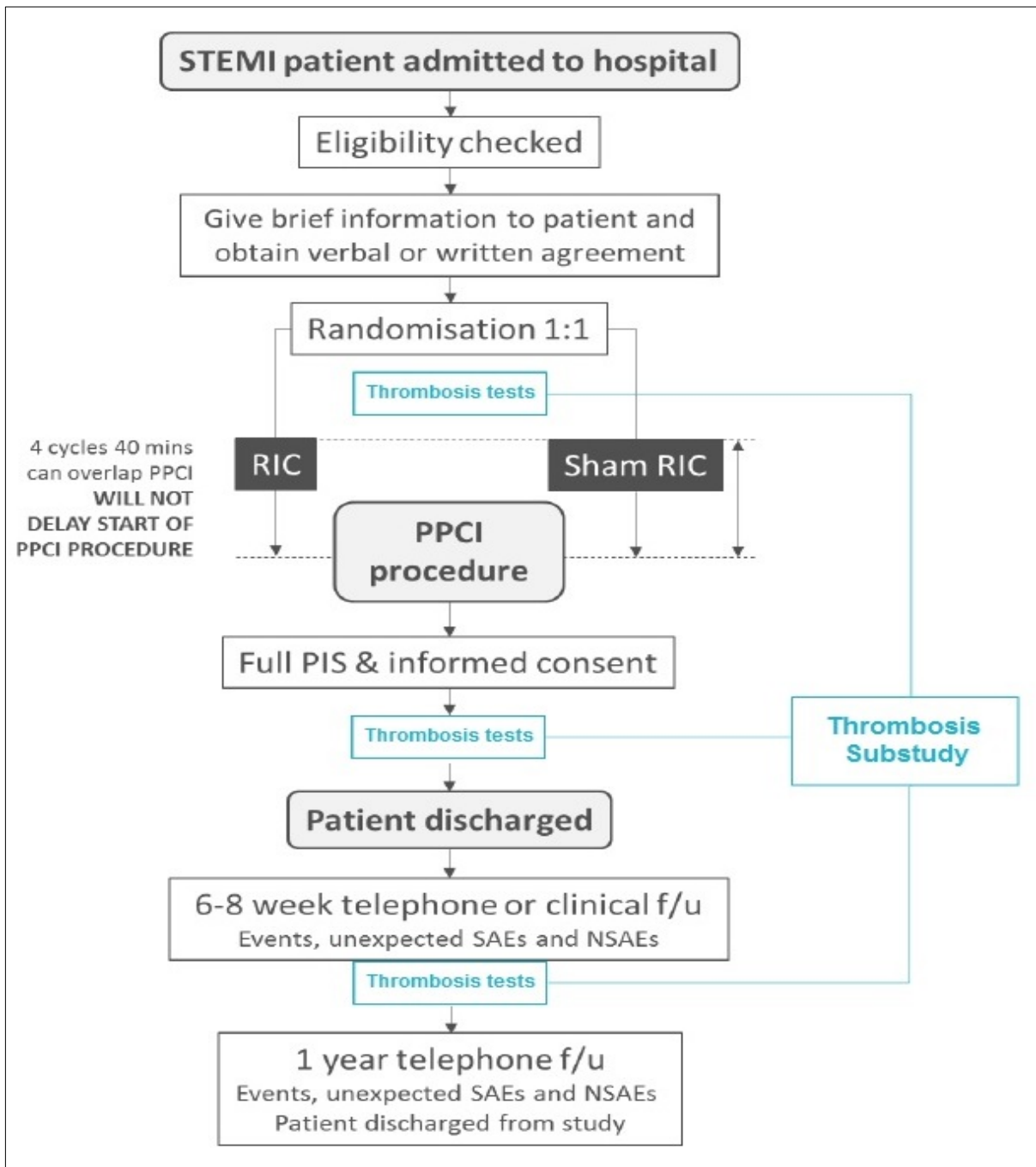
743

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

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



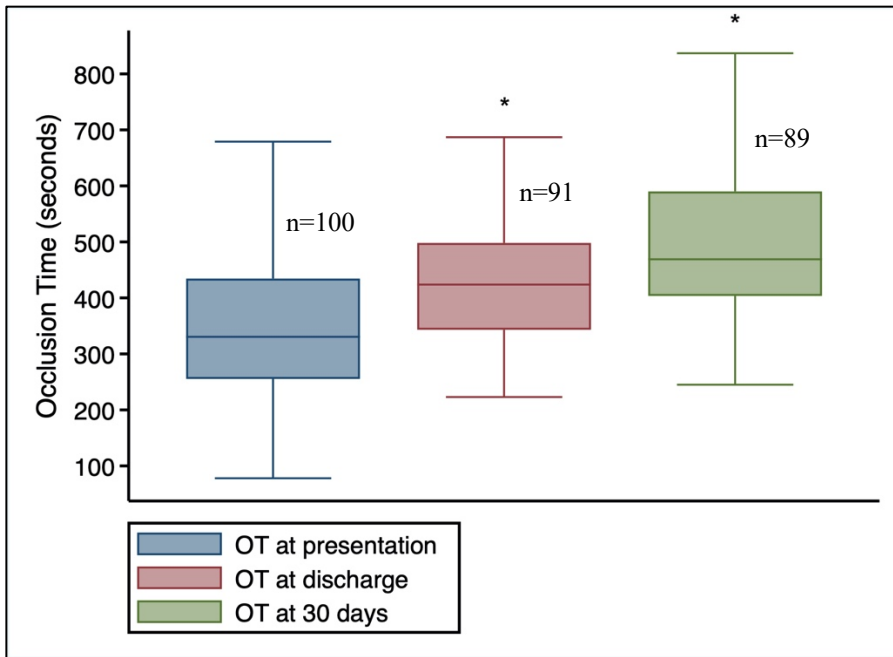
747 **Figure 1.**



748

749 **Figure 2.**

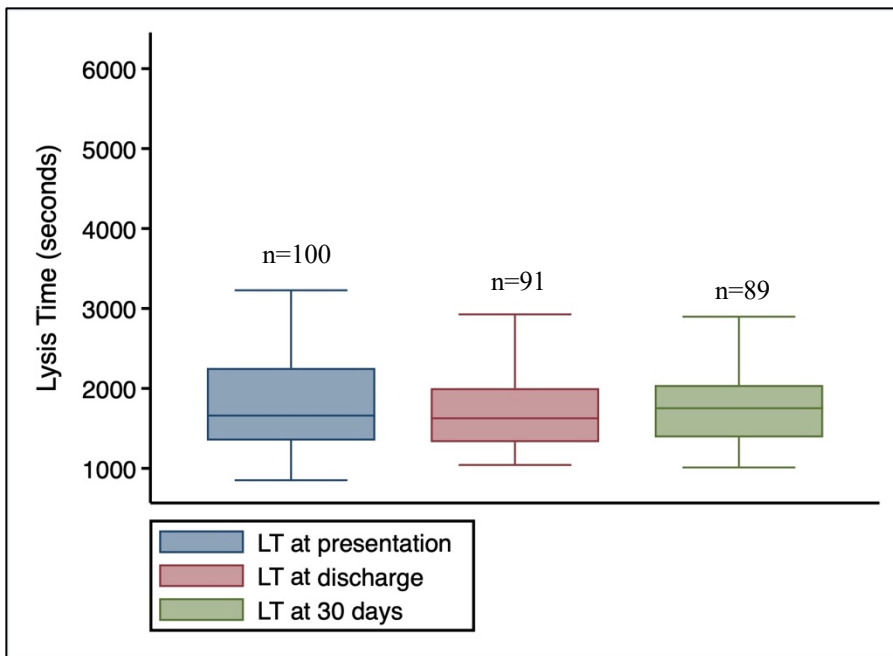
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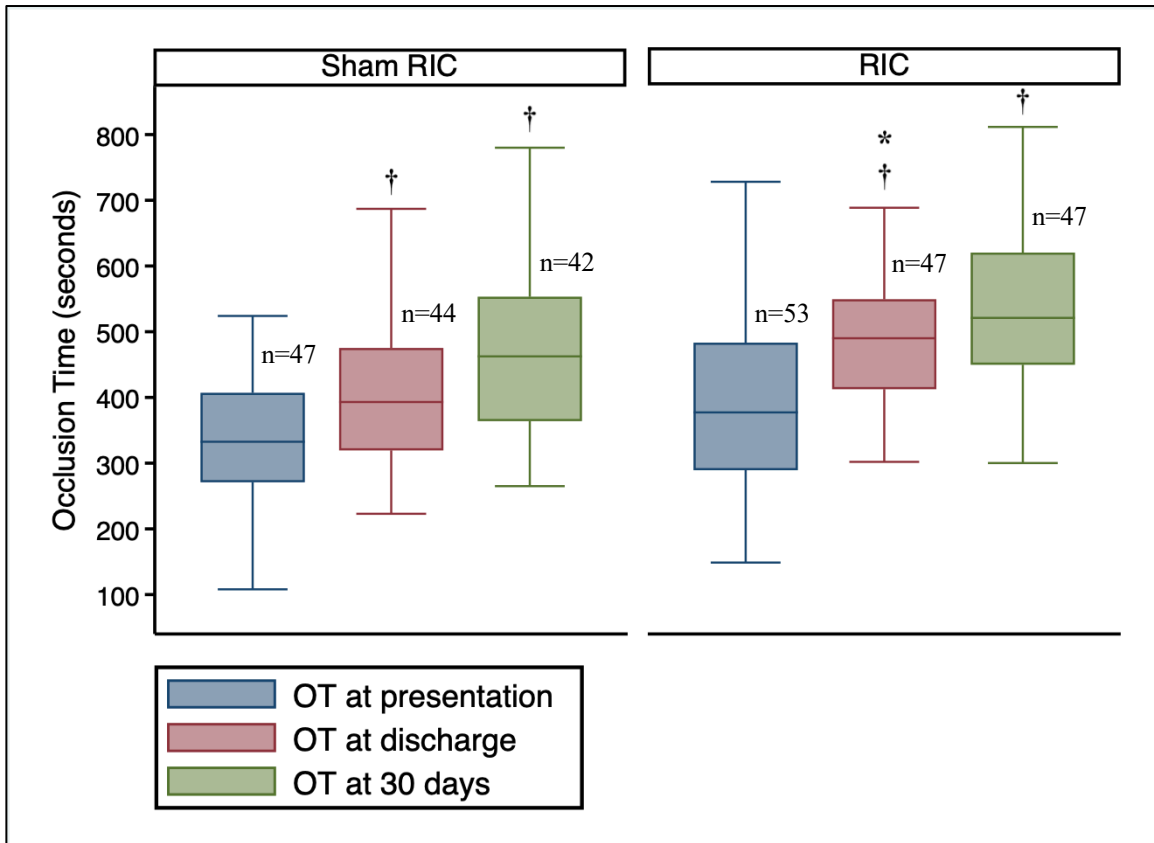
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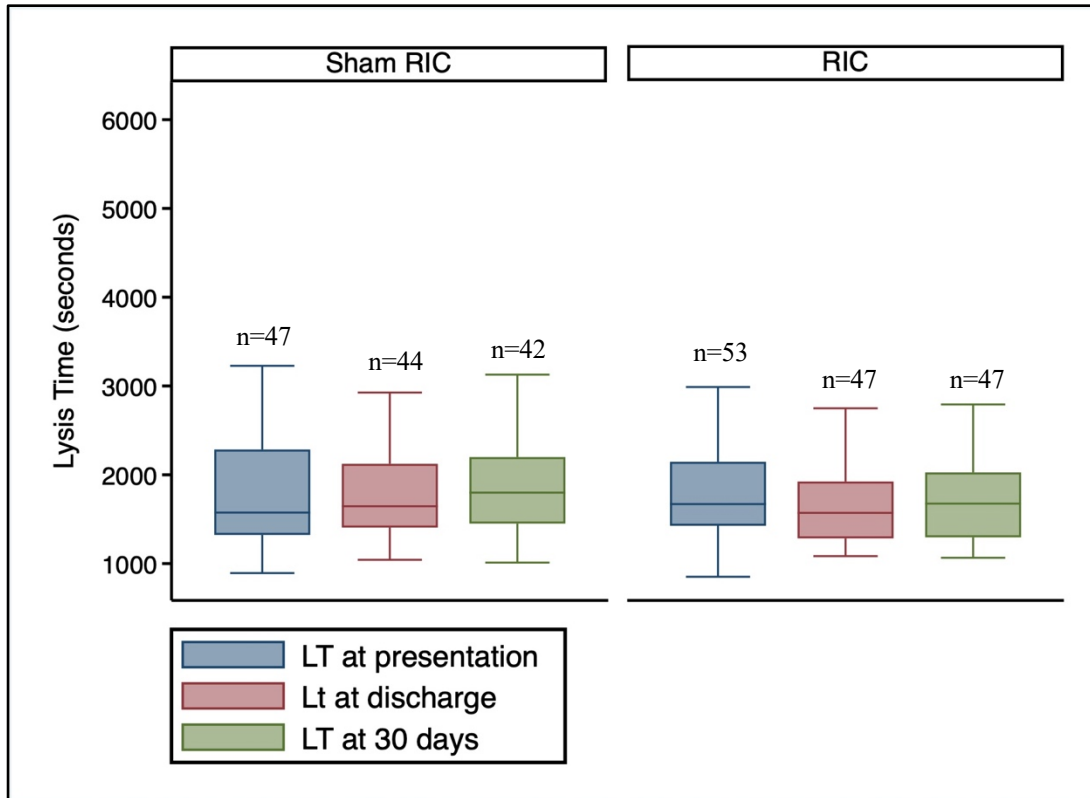
755

756 **Figure 3.**



757

758 **Figure 4.**



759

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 <sub>12</sub> inhibitor use	1 (1.0)	0	1 (1.9)	1.000
<b>Initial P2Y<sub>12</sub> inhibitor loading agent</b>				
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 <sub>12</sub> inhibitor loading to first blood sample (min)	46.9±21.9	46.9±19.1	46.9±24.2	0.979
<b>Medications prior to hospital discharge</b>				
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

764 Values are mean  $\pm$  standard deviation or n (%). Renal insufficiency was defined as creatinine levels  $>177 \mu\text{mol/L}$ .  
765 Prior statin, aspirin or P2Y<sub>12</sub> inhibitor use defined as regular statin, aspirin or P2Y<sub>12</sub> 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**

	<b>Whole Group (n=100)</b>	<b>Sham RIC (n=47)</b>	<b>RIC (n=53)</b>	<b>P Value</b>
<b>Haemoglobin (g/L)</b>	138±19	136±19	139±19	0.400
<b>Haematocrit (%)</b>	41±6	40±6	41±5	0.516
<b>Neutrophil count (x10<sup>9</sup>/L)</b>	8.6±2.9	8.6±2.8	8.6±3.1	0.938
<b>Platelet count (x10<sup>9</sup>/L)</b>	259±77	258±78	260±77	0.923
<b>Serum albumin (g/L) *</b>	43±3.7	42±3.8	43±3.7	0.243
<b>Sodium (mmol/L)</b>	138±3	138±2	138±3	0.789
<b>Creatinine (µmol/L)</b>	91±37	94±49	89±23	0.513
<b>Peak troponin T (ng/L) *</b>	2223 [1072-3796]	2014 [993-3606]	2301 [1074-3945]	0.474
<b>Fibrinogen (g/L)</b>	4.6±1.3	4.6±1.1	4.7±1.5	0.605
<b>PT (sec)</b>	11.8±1.1	11.8±1.0	11.9±1.2	0.728
<b>aPTT (sec)</b>	28.1±3.6	27.5±3.4	28.6±3.7	0.175
<b>Total cholesterol (mmol/L)</b>	5.1±1.2	4.9±1.2	5.3±1.1	0.121
<b>LDL cholesterol (mmol/L)</b>	2.9±0.8	2.9±0.9	2.9±0.7	0.867
<b>Hs C-reactive protein (mg/l) *</b>	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<sup>9</sup>/L; platelet count 150-400 x10<sup>9</sup>/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**

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

784

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

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

795 Table 5. Tests of thrombotic status

	Whole Group (n=100)	Sham RIC (n=47)	RIC (n=53)	P value
<b>Global Thrombosis Test (GTT)</b>				
<i>Baseline</i>				
OT [sec] *	337±129	329±98	349±151	0.444
LT [sec]	1660[1348-2255]	1574[1323-2284]	1670[1426-2146]	0.777
<i>At discharge</i>				
OT [sec] *	430±107	403±105	454±105	0.025
LT [sec]	1626[1328-2002]	1646[1406-2123]	1571[1284-1924]	0.241
<i>At 6-8 weeks</i>				
OT [sec] *	493±132	471±132	512±130	0.144
LT [sec]	1752[1387-2042]	1799[1451-2199]	1675[1296-2026]	0.227
<b>Thromboelastography (TEG)</b>				
<i>Baseline (native blood sample)</i>				
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
<i>At discharge (native blood sample)</i>				
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

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

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

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

799 Table 2.

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