Remote Ischemic Postconditioning of the Lower Limb During Primary PCI Safely Reduces Enzymatic Infarct Size in Anterior Myocardial Infarction: a Randomized Controlled Trial

Gabriele Crimi, MD†*, Silvia Pica, MD*, Claudia Raineri, MD*, Ezio Bramucci, MD*, Gaetano M. De Ferrari, MD*, Catherine Klersy, MD, MSc‡, Marco Ferlini, MD*, Barbara Marinoni, MD*, Alessandra Repetto, MD*, Maurizio Romeo, MD†, Vittorio Rosti, MD§, Margherita Massa, PhD§, Arturo Raisaro, MD*, Sergio Leonardi, MD, MHS*, Paolo Rubartelli, MD†, Luigi Oltrona Visconti, MD*, Maurizio Ferrario, MD*.

*SC. Cardiologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; †SC. Cardiologia, ASL3 Ospedale Villa Scassi, Genova, Italy; ‡Servizio Biometria e Statistica Direzione Scientifica, IRCCS Policlinico San Matteo, Pavia, Italy; §Laboratorio di Biotecnologie, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

The authors have no relationship with industries to disclose regarding the article. This study was funded by a research grant of the Fondazione IRCCS Policlinico San Matteo, Pavia.

Address for correspondence:

Gabriele Crimi, MD
SC Cardiologia, ASL3 Ospedale Villa Scassi,
Corso Scassi 1,
16149 Genoa, Italy,
Phone +39 010 8492663 , Fax +39 010 8492270
Email: gabrielecrimi@gmail.com

Abstract

Objective

To evaluate whether remote ischemic postconditioning (RIPC) could reduce enzymatic infarct size in patients with anterior ST-elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (pPCI).

Background

Myocardial reperfusion injury (MRI) may attenuate the benefit of pPCI. In animal models RIPC mitigates MRI.

Methods

One hundred patients with anterior STEMI and occluded left anterior descending artery were randomized to pPCI + RIPC (n=50) or conventional pPCI (n=50). RIPC consisted of 3 cycles of 5'/5' ischemia/reperfusion by cuff inflation/deflation of the lower limb. The primary endpoint was infarct size assessed by the area under the curve of creatinine kinase-MB release (AUC CK-MB). Secondary endpoints included infarct size assessed by cardiac magnetic resonance (CMR) delayed enhancement (DE) volume; T2-weighted (T2W) edema volume; ST-segment resolution >50% (STR_{50}); TIMI frame count (TIMI-FC); and myocardial blush grading (MBG).

Results

Four patients (2 RIPC, 2 controls) were excluded due to CK-MB missing samples. 96 patients were analyzed; median AUC CK-MB was 8814 arbitrary units (interquartile range, 5567 to 11325) in the RIPC group and 10065 arbitrary units (interquartile range, 7465 to 14004) in controls (relative reduction of 20%, 95%CI, 0.2% to 28.7%, p=0.043). 77 patients underwent a CMR scan 3 to 5 days after randomization and 66 patients repeated a second scan after 4 months. T2W edema volume was 37 ± 16 cc in RIPC patients and 47 ± 22 cc in controls (p=0.049). STR_{50} was 66% in RIPC and 37% in controls (p=0.015). We observed no significant differences in TIMI-FC, MBG and DE volume.

Conclusions

In patients with anterior STEMI, RIPC at the time of primary PCI reduced enzymatic infarct size and was also associated with an improvement of T2W edema volume and STR_{50}.

Key Words

Myocardial infarction, myocardial reperfusion injury, primary angioplasty, cardiac magnetic resonance imaging, myocardial conditioning.

Abbreviations list

STEMI = ST-elevation myocardial infarction

pPCI = primary percutaneous coronary intervention

RIPC = remote ischemic postconditioning

LAD = left anterior descending artery

ce-CMR = contrast enhanced cardiac magnetic resonance

T2W = T2-weighted

anti-GP IIb-IIIa = inhibitors of glycoprotein IIb-IIIa

CK-MB = creatine kinase myocardial band

STR = ST-segment resolution

LV = left ventricle/ventricular

ST-elevation myocardial infarction (STEMI) is a leading cause of mortality and morbidity worldwide. Infarct size is a key determinant of prognosis (1). Timely and successful reperfusion, best achieved with primary percutaneous coronary intervention (pPCI), is effective at reducing infarct size, preserving ventricular function and improving outcome (2). Nevertheless, abrupt restoration of blood flow carries itself a lethal injury on myocardial cells that may limit the benefit of such intervention. In pre-clinical studies, the impact of myocardial reperfusion injury accounts up to 50% of the final infarct size (3). Among strategies aimed at limiting reperfusion injury, myocardial ischemic postconditioning, obtained by exposing the ischemic myocardium to brief periods of ischemia/reperfusion immediately after reperfusion, showed promising results in both animal (4) and small clinical studies (5-9). However, the relevance of this intervention in the clinical setting remains unclear (10) and more importantly, safety of repeated balloon inflations into the infarct related artery has been recently questioned (11, 12).

In 1993 Przyklenk et al. (13) experimentally proved that the cardioprotective effect of myocardial conditioning could be also elicitated by brief ischemia/reperfusion cycles applied remotely. This form of myocardial conditioning, termed *remote conditioning*, has shown protective effects (14), even more relevant than *local* conditioning in animals (15). Bøtker et al. tested remote ischemic conditioning in patients with STEMI (16). They observed an improvement in myocardial salvage, although a reduction of infarct size was only evident in the explorative subgroup analysis of patients with STEMI due to occluded left anterior descending artery (LAD).

The objective of our study was to evaluate whether remote ischemic postconditioning (RIPC) could safely reduce enzymatic infarct size in patients with anterior STEMI treated with pPCI within 6 hours of symptoms onset. To test this hypothesis we randomized patients with occluded LAD to pPCI + RIPC or pPCI alone. We also evaluated markers of reperfusion injury such as contrast enhanced cardiac magnetic resonance (ce-CMR) T2-weighted (T2W) edema (17) and micro-vascular perfusion (angiographic and ECG parameters).

Methods

Study population

Patients aged 18 to 80 years, presenting within 6 hours of symptoms onset, with anterior STEMI, *de-novo* occlusion of LAD (TIMI flow 0-1) and planned pPCI were eligible. Anterior STEMI was defined as the occurrence of >20 minutes chest pain, ST-segment elevation (>2 mm) in at least 2 contiguous precordial leads. Exclusion criteria were: previous anterior STEMI or ≤6 months non-anterior STEMI; Killip class IV; evidence of retrograde filling by collaterals at coronary angiography; severe multivessel coronary artery disease likely to require further interventions before follow-up ce-CMR (4 months); known severe abdominal aortic aneurysm (>50 mm) or severe peripheral artery disease (class III-IV).

All patients were followed for 1 year with an office visit at 4 months and a telephone call at 12 months. During follow-up we assessed vital status, recurrent myocardial infarction, repeated myocardial revascularizations, and stroke.

Study Design

This was a randomized, controlled, parallel-groups, open label trial, with blinded evaluation of the end points. The study was conducted in two centers in Italy. Randomization was generated via computer-assisted sequence of treatments with permuted blocks of varying size, using sealed opaque envelopes. After diagnostic angiography, eligible patients were randomized 1:1 to pPCI + RIPC or conventional pPCI (Figure 1). All patients were pre-treated with 250 mg aspirin intravenously, 300 or 600 mg clopidogrel loading dose and 70 IU/Kg unfractioned heparin. Coronary angiography was performed either by femoral or radial approach. Both groups received pPCI according to standard techniques; the use of thrombectomy and of inhibitors of glycoprotein IIb-IIIa (anti-GP IIb-IIIa) was strongly encouraged. Infusion of anti-GP IIb-IIIa was prolonged for 12h or 18h as appropriate. Lifelong 100 mg aspirin, ACE-inhibitors, beta-blockers statins were prescribed daily, 75 mg clopidogrel daily was prolonged for 12 months.

Data were collected, analyzed and the manuscript was written solely by the authors who are responsible for its content. The trial was performed according to the Declaration of Helsinki (revised

version, 1996) and the European Guidelines for Good Clinical Practice (version 11, July 1990). The study was approved by local ethic committees. All patients signed a written informed consent. The study was registered with ClinicalTrials.gov, #NCT00865722.

Remote ischemic postconditioning protocol

All eligible patients were prepared with a thigh-sized limb cuff before arterial puncture (contralateral in case of femoral access). In the active treatment group, the protocol was started with thrombectomy or balloon inflation whichever occurred first. The lower limb was exposed to 3 cycles of ischemia/reperfusion, each obtained by 5 minutes cuff inflation at 200 mmHg, followed by 5 minutes complete deflation (Figure 1). Study personnel involved in RIPC administration (nurses, fellows or physicians) was not involved in the assessment of the endpoints.

Endpoints

Enzymatic infarct size

Primary endpoint was the enzymatic infarct size assessed by the area under the curve of creatine kinase myocardial band (CK-MB) release (18). Blood samples were collected before PCI, every 6 hours during the first 48 hours, and at 72 hour. Blood was processed and analyzed locally using the Abbott Architect immunoassay for CK-MB. Area under the curve of CK-MB release was expressed in arbitrary units and calculated with the trapezoidal method $1/2\sum_{t=0}^{t=72}(CKMBt0+CKMBtx)\Delta t$.

TIMI frame count and myocardial blush grading

TIMI frame count and myocardial blush grading (MBG) were evaluated 30 min after the first balloon inflation or thrombectomy in both the study arms. TIMI frame count was the number of frames necessary to fill LAD to the distal bifurcation (19). MBG was defined as previously described (20). MBG for LAD was evaluated in left lateral projection and compared with the one of right coronary artery.

ST-segment resolution

ST-segment elevation was evaluated by a single investigator with lens-intensified caliper to the nearest of 0.5 mV, 20 msec after the end of QRS. PR segment was reference baseline. ECGs were collected at admission and ≈60 minutes after reperfusion (time window ± 10 minutes). The lead with maximal ST-elevation at baseline was analyzed. ST-segment resolution (STR) was expressed as percentage of change from baseline; cutoffs were set at 50% (STR_{50}) and 70% (STR_{70}) as previously described (21).

Contrast-enhanced cardiac magnetic resonance

Acquisition protocol

ce-CMR was performed 3-5 days after randomization and after 4 months. 1.5-T MAGNETOM Symphony (Siemens Healthcare, Erlangen, Germany) with a phased array receiver-coil on the patient chest was used. All subjects were placed in supine position, left ventricular (LV) function was assessed using breath-hold, steady-state, free precession cine-CMR. Contiguous slices were acquired from atrio-ventricular plane to apex in short-axis direction. Imaging parameters were: time to repeat (TR) = 46.41 msec, time of echo (TE) = 1.79 msec, flip angle (FA) = 65^{o}, slice thickness (ST) = 8 mm, matrix = 166 x 256, field of view (FOV) = 280 to 350 mm. T2W images were acquired in long-axis and short-axis direction from base to apex using dark-blood T2W short tau inversion-recovery (STIR) fast spin-echo (FSE) sequences. Imaging parameters were: TR = 750 to 2000 msec, TE = 69 msec, FA = 180^{o}, ST = 8 mm, matrix =128 x 256, FOV = 300 to 380 mm. Intravenous bolus of gadolinium with diethylenetriaminepentacetate (Magnevist, Schering, Berlin, Germany) 0.2 mmol/kg was administered. Early (≈5 minutes) contrast images were used to evaluate microvascular obstruction; late images (≈15 minutes) were used to evaluate infarct size. Images were acquired in short and long axis with T1-weighted inversion-recovery sequences (inversion time 240 to 300 msec).

Image analysis

Two senior investigators computed parameters off-line (Argus, Siemens Medical solutions, Malvern, Pennsylvania); disagreement was solved by consensus. LV endocardial and epicardial borders were

manually traced. Each short-axis slice at both end-diastole and end-systole was evaluated. Myocardial mass was calculated by multiplying myocardial volume by tissue density (i.e. 1.05 g/mL). The hyperintense signal in T2W STIR FSE images was considered for myocardial edema. Edema volume was computed including signal at least two standard deviations above the mean of non-infarcted myocardium. Hyperintense signal from the blood pool was excluded. Myocardial hemorrhage was defined as a hypointense signal within the area of edema. Hemorrhage was included in computation of T2W edema volume. Infarct size was assessed on late-contrast images (≈15 minutes after gadolinium administration) by manual tracing the hyperintense area in each short-axis slice. Microvascular obstruction was defined as a hypointense signal in the infarct-related myocardium and was assessed by manual tracing the suspected area in each short-axis slice.

Sample size

At the time of the study design, limited clinical data were available for sample size estimation (5). We conservatively planned to enroll a total of 100 patients (50 per group) to obtain 92 valid patients (power 90%, alpha 2-sided 5%, effect size 0.678, corresponding to a mean area under the curve of 326095 in the control group and of 241400 in the treatment group, with a common standard deviation of 125000). Two interim analyses were performed for efficacy and adaptation of sample size after the enrollment of 40 and 60 patients, respectively (Lan-DeMets group sequential method, O'Brien-Fleming type alpha spending function). To preserve an overall alpha of 0.050, the p-values to reject the null hypothesis of no difference between treatments at the interim analyses was computed to 0.0005 and 0.014, respectively and 0.045 at the final analysis (22).

Calculations were performed with WinL (Programs for Computing Group Sequential Boundaries Using the Lan-DeMets Method. Version 2. by DM. Reboussin, DL DeMets and KKG Lan) and Stata 12 (StataCorp, College Station, TX, USA).

Statistical analysis

Data were described as mean and standard deviation or median and 25^{th}-75^{th} percentiles if continuous and counts and percent if categorical. The log-transformed primary outcome measure was compared

between groups with the Student t test. The difference between groups was then back-transformed and presented as ratios of control vs. treatment, with 95% confidence interval (95%CI). Secondary outcome measures were compared with the Fisher exact test if categorical and either the Student t test or the Mann Whitney U test (depending on their distribution) if continuous. Association between continuous variables was measured with the Spearman R. Finally an exploratory analysis of the primary endpoint to verify whether differentials effects of treatment were present in predefined subgroups of patients (age, gender, diabetes mellitus, pre-infarctual angina, morphine administration, pain to balloon time or presence of a multivessel disease) by fitting a multiple general linear model with inclusion of an interaction term between treatment and subgroup. Model assumption was verified by inspection of residuals.

Stata12 (StataCorp, College Station, TX, USA) was used for computation. A 2-sided P-value<0.05 was considered statistically significant.

Results

Patients

Between March 2009 and December 2011, 753 pPCI were performed, including 224 anterior STEMI. The main reason for study ineligibility was severe multivessel disease likely to require staged PCI (Figure 2) (23). We enrolled 100 patients. Four patients (2 RIPC and 2 controls) had missing blood samples for primary endpoint assessment and were excluded. The primary analysis included 96 patients. Baseline characteristics were balanced between the study groups (Table 1 and eTable 1). Mean age was 58.4 ± 10.9 years, 88% patients were males, 12% diabetics, 63% had single-vessel disease. Median ischemia time (pain to balloon) was 182 minutes (interquartile range: 145 to 239 minutes) with no differences between the study groups. Overall, 96% of patients were treated with anti-GP IIb-IIIa and 81% with thrombectomy. Morphine (5 to 10 mg IV boluses) was administered before or during the procedure in 57% of patients.

Enzymatic infarct size

The median area under the curve of CK-MB release was 8814 arbitrary units [interquartile range 5638 to 11322 arbitrary units] in RIPC patients and 10065 arbitrary units [interquartile range 7510 to 13980 arbitrary units] in controls (Figure 3). This represents a reduction of 20% (95% CI, 0.2% to 28.7%, p=0.043).

Angiographic endpoints

TIMI frame count was similar between RIPC and controls groups. The proportion of patients with myocardial blush grade 2 or 3 was numerically higher in patients randomized to RIPC (Table 3). Final TIMI flow 2-3 was achieved in 98% of RIPC patients and 96% of controls (P=0.554).

ECG endpoints

STR was significantly improved in RIPC patients compared to controls (Table 3). Consistent results were obtained in RIPC group assessing either STR_{50} and STR_{70}.

ce-CMR endpoints

During hospitalization ce-CMR was performed in 35 (73%) RIPC patients and 42 (87%) controls. Thirty patients (87%) in the RIPC group and 36 controls (87%) repeated ce-CMR after 4 months. Patients who did not perform ce-CMR had similar baseline characteristics than those who did in terms of age, gender, diabetes, pain to balloon time, segment of LAD occlusion, single vs. multi-vessel disease and area under the curve of CK-MB release (data not shown).

Detailed description of all the parameters is outlined in Table 4 and eTable 2. In RIPC patients, compared with controls, there was a significant 20.6% relative reduction of T2W edema volume (95%CI, 2.6% to 42.2% p=0.049) Figure 4. Myocardial edema correlated well with both acute delayed gadolinium enhancement (DE) volume (R=0.88, P<0.001) and DE volume at follow-up (R=0.70, P<0001; figure 2). We observed a numerical, difference in favor of RIPC in acute DE volume (relative reduction: 20.7%, 95%CI, -2.3% to 39.1%) and LV ejection fraction. After 4 months follow up the relative reduction of DE volume in the RIPC group was 10.0% (95%CI, -16.1% to 37.5%) Acute ce-CMR DE volume (R=0.70, p<0.001), T2W edema volume (R=0.65, p<0.001) and

follow-up ce-CMR DE volume (R=0.66, p<0.001) correlated well with enzymatic infarct size (eFigure 1). Based on the observed effect size, the *post-hoc* power analysis to detect a difference in acute and follow up DE volume were respectively 1-β=0.46 and 1-β=0.10.

Subgroup analysis

In subgroup analysis, no significant interaction was observed between RIPC and the pre-specified subgroups: age (<60 vs ≥60 years), gender, diabetes mellitus, pre-infarction angina, morphine administration, pain to balloon time (tertiles) and single vs multivessel disease.

Secondary clinical endpoints and adverse events

RIPC was successfully administered to all patients. Major adverse cardiac events are outlined in Table 2. One patient died in the control group (refractory heart failure) and none in the RIPC group. In the control group we observed 1 re-infarction due to subacute stent thrombosis requiring re-PCI, and 1 ischemic stroke. In the RIPC group we observed 4 repeated coronary revascularization (3 treated with PCI, 1 with CABG). Vital status was assessed after 1-year follow-up in all but 1 patient who moved to another country (RIPC group).

Discussion

Our study shows that RIPC of the lower limb at the time of pPCI, can reduce enzymatic infarct size by 20% (95%CI, 0.2% to 28.7%) in patients with anterior STEMI and occluded LAD, undergoing pPCI within 6 hours of symptoms onset. The benefit observed, though smaller than expected and marginally significant, was directionally consistent with markers of myocardial reperfusion injury and successful reperfusion such as a reduction of T2W edema volume and an improvement in STR. Notably these effects were observed in addition to those provided by optimal thrombus management including thrombectomy and use of anti-GP IIb-IIIa.

Most of the clinical studies that explored the effect of myocardial postconditioning in humans, applied brief cycles of ischemia/reperfusion to the culprit artery after stenting. The protective effect of *local* postconditioning in STEMI patients remains unclear and prompted safety concerns related to

possible thrombus microembolization occurring during repeated balloon inflations (12) in the infarct-related artery.

To date, there are only two studies exploring the effects of *remote* conditioning in patients undergoing pPCI (16, 24). However, this is the first study to assess the effects of *remote post*conditioning since, at variance with the previous ones, ischemia and reperfusion of a remote district (the lower limb) was initiated only after coronary reperfusion. Botker et al. (16) studied remote ischemic conditioning performed during hospital transportation. The primary endpoint was myocardial salvage assessed by myocardial perfusion imaging. Myocardial salvage was improved in remote conditioned patients, but no effects on infarct size, STR and troponin-T release were observed.

Rentoukas et al. (24) tested the effects of remote ischemic conditioning initiated prior to pPCI combined with morphine administration in patients with STEMI. They enrolled 96 STEMI patients presenting within 6 hours of symptoms onset randomly assigned to one of three reperfusion strategies (RIPC + pPCI, RIPC + pPCI + morphine, pPCI alone). The primary endpoint of full STR was reduced in both RIPC + pPCI and RIPC + pPCI + morphine groups.

Two relevant aspects, regarding the remote ischemia/reperfusion protocol applied to elicitate cardioprotection makes our study original: *timing* and *site*.

Timing. Bøtker et al. (16) completed 4 cycles of remote ischemia/reperfusion *before* pPCI. This protocol differs from the original concept of *post*conditioning described by Zhao et. al. (4) since it was initiated after the onset of ischemia, but before reperfusion. This strategy could be defined as a remote *late pre*conditioning. Rentoukas et al. (24) started 3 cycles of remote ischemia/reperfusion 10 minutes before the estimated time of reperfusion and continued 5 minutes after. Infact they termed it "remote *peri*conditioning". We designed the present study to closely translate the pre-clinical model (14) of remote *post*conditioning into clinical practice. We randomized patients with occluded LAD without evidence of retrograde filling to avoid potential confounders due to spontaneous reperfusion and/or collateral protection. RIPC consisted of 3 cycles of leg ischemia/reperfusion started *at the time* of reperfusion. We believe that timing could have potentially relevant implications beyond classification. In particular, we hypothesize that any circulating factor potentially mediating the

cardioprotective effects (e.g. pH shifts, endogenous adenosine, opioids, cytokines) has to reach the target area (i.e. ischemic myocardium), thus requiring a patent culprit artery.

Site. Both the previous studies applied ischemia/reperfusion cycles to one arm. A dose-response effect, in regard with both site and number of cycles of remote ischemia/reperfusion was elegantly demonstrated by Loukogeorgakis SP and collegues (25). They explored the protective effect of remote conditioning against endothelial ischemia/reperfusion injury of the arm. They assessed flow-mediated dilatation before and after 20 min of ischemia of the arm obtained with extrinsic compression. A dose-response protective effect was reported, with a maximum degree obtained with 3 cycles of ischemia/reperfusion of the leg and a threshold effect of at least two cycles of the arm. According to these data we hypothesized a dose-response effect proportional to the mass of the remote district and we choose the lower limb, which represents approximately two times the body surface and even more tissue volume of the upper limb.

Another potentially relevant issue, compared with previous studies, is thrombus management. In the animal model coronary occlusion is obtained in the absence of thrombus. In the clinical setting, the presence of thrombus and potential distal embolization might be important confounder. For this reason the use of both anti-GP IIb-IIIa and thrombectomy (95% and 84% respectively) was strongly encouraged in our study. In the study by Bøtker et al. (16) anti-GP IIb-IIIa were administered in ≈84% of patients, while these data were not reported by Rentoukas et al.(24) and the use of thrombectomy devices was neither reported.

Infarct size reduction observed in RIPC patients with enzymatic CK-MB release was less evident when assessed by ce-CMR imaging. DE volume was non-significantly reduced in RIPC patients. The reason for this difference is unclear. There was a good correlation between enzymatic infarct size and DE volume in both the acute phase and the follow-up and the point estimate of the mean infarct size reduction was similar using enzymatic and DE volume criteria (Figure 4). However, DE volume was measured in 80% of patients. So it could be possible that the lack of difference was due to insufficient power. Furthermore, the small improvement in DE volume and LV ejection fraction associated with RIPC in the acute phase was no longer evident at 4 months. This finding, similar to that reported by Bøtker (16) as well as by other studies testing local postconditioning (11,

12) could be the result of infarct size shrinkage and left ventricle remodeling that further reduces power during follow-up.

The results of secondary endpoints support the hypothesis that RIPC could reduce infarct size by limiting myocardial reperfusion injury in humans.

RIPC was associated with a reduction of myocardial T2W edema volume, a potential marker of reperfusion injury (17). Edema starts during the ischemic phase and expands in the interstitial space during reperfusion, thus potentially increasing hydrostatic pressure and causing capillary compression. Myocardial edema could have an indirect negative effect on myocardium and contribute to the pathogenesis of reperfusion injury (27, 28).

RIPC significantly improved STR. STR has been proposed as a marker of efficient microvascular reperfusion and it yields prognostic information beyond that provided by coronary TIMI flow. Several studies have shown consistent relationship between STR and subsequent mortality (29).

RIPC was also associated with a trend of improvement in MBG, while no difference was found in TIMI frame count. MBG has been proposed as a more efficient marker of successful microvascular reperfusion than TIMI flow and TIMI frame count and has been positively associated with long-term mortality (20) in STEMI patients.

RIPC was easy to perform, inexpensive, and well tolerated. We observed no adverse effect related to RIPC during hospitalization. No patient died in the RIPC group at 12 months. During follow-up we observed 4 repeated revascularization in RIPC patient. These clinical events were due to in-stent restenosis following bare metal stent implantation, so the relationship with the study treatment is unlikely.

Study limitation

The study was not double-blinded. However, to limit potential sources of bias, all patients were prepared with tight-sized cuff and the endpoints were evaluated by investigators blinded to treatment allocation.

The results reflect a selected population of patients with STEMI. Moreover, the ratio between patients screened and randomized was higher and the period of enrollment was longer than originally planned. However, no major changes were observed in the managements of patients with STEMI during this time, so it is likely that the impact of this aspect is limited.

We did not assess the area at risk, nevertheless, we enrolled only patients with occluded LAD and the segment of occlusion, a surrogate of the area at risk, was not different between the groups.

The study was not adequately powered to detect a difference in DE volume both in the acute phase and at follow-up.

Conclusions

RIPC of the lower limb at the time of primary PCI in patients with anterior STEMI due to occluded LAD within 6 hours of symptoms onset, reduces enzymatic infarct size and is associated with the improvement of markers of myocardial reperfusion injury (T2W edema volume) and microvascular reperfusion (STR). Given its excellent feasibility, safety profile and minimal costs, these encouraging results warrant further investigations.

Acknowledgements

The authors are grateful to the people that made this study possible: Bobo Moroni, RN, Paolo Roversi, RN, Piera Allara, RN, Giovanna Avenoso, RN, Marina Cervio, RN, Marinella Damo, RN, Cristina Frattini, RN, Gloria Lazzarini, RN, Lorena Maldarelli, RN, Massimo Mariconti, RN, Giusy Orlandi, RN, Grazia Ziraldo, RN, Luigi Carlo Bottaro, MD, Davide Bartolini, MD, Sandro Bellotti, MD, Alessandro Iannone, MD. We are also thankful to the participating patients who volunteered to help others.

References

1. Gwilt DJ, Petri M, Lewis PW, Nattrass M, Pentecost BL, Myocardial infarct size and mortality in diabetic patients. *Br Heart J*. 1985;54:466-472.
2. Zijsta F, Hoorntje JCA, Jan De Boer M, et al. Long-term benefit of primary angioplasty as compared with thrombolytic therapy for acute myocardial infarction. *N Eng J Med*. 1999,341:1413-1419.
3. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med*. 2007;357:1121-1135.
4. Zhao ZQ, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Phisiol*. 2003;285:H579-H588.
5. Staat P, RioufolG, Piot C, et al. Postconditioning the human heart. *Circulation* 2005;112:2143-2148.
6. Ma X, Zhang X, Li C, Luo M. Effect of postconditioning on coronary blood flow velocity and endothelial function and LV recovery after myocardial infarction. *J Interv Cardiol*. 2006;19:367-375.
7. Laskey WK, Yoon S, Calzada N, Ricciardi MJ. Concordant improvements in coronary blood flow reserve and ST-segment resolution, during percutaneous coronary intervention: a benefit of postconditioning. *Catheter Cardiovasc Interv*. 2008;72:212-220.
8. Yang XC, LIU Y, Wang LF, et al. Reduction in myocardial infarct size by postconditioning patients after percutaneous coronary interventions. *J Invasive Cardiol*. 2007;19-424-430.
9. LøngborgJ, Kelbaek H. Vejlstrup N, et al. Cardioprotective effect of ischemic postconditioning in patient treated with primary percutaneous coronary intervention, evaluated by magnetic resonance. *Circ Cardiovasc interv*. 2010;3:34-41.
10. Sörennson P, Saleh N, Bouvier F, et al. Effect of postconditioning on infarct size in patients with ST elevation myocardial infarction. *Heart*. 2010;96:1710-1715.

11. Freixa j, Bellera N, Ortiz-Pérez JT, et al. Ischaemic postconditioning revisited: lack of effect on infarct size following primary percutaneous coronary intervention. *Eur Heart J*. 2012;33:103-112.
12. Tarantini G. Favaretto E, Perazzolo Marra M, et al. Postconditioning during coronary angioplasty in acute myocardial infarction: the POST-AMI trial. *Int J Cardiol*. 2012;162(1):33-8.
13. Przyklenk K, Bauer B, Ovize M, Kloner R, Whittaker P. Regional ischemic 'Preconditioning' protects remote virgin myocardium from subsequent sustained occlusion. *Circulation*. 1993;87:893-899.
14. Andreka G, Vertesaljai M, Szantho G, et al. Remote ischaemic postconditioning protects the heart during acute myocardial infarction in pig. *Heart*. 2007;93(6):749-752.
15. Gritsopoulos G, Iliodromitis EK, Zoga A, et al. Remote postconconditioning is more potent than classic postconditioning in reducing infarct size in anesthetized rabbits. *Cardiovasc Drug Ther*. 2009; 23:193-198.
16. Bøtker HE, Kharbanda R, Schmidt MR, et al. Remote ischemic conditioning before hospital admission as a complement to angioplasty, and effect on myocardial savage in patients with acute myocardial infarction: a randomized trial. *The Lancet*. 2010;375:727-734.
17. Thuny F, Lairez O, Roubille F, et al. Post-conditioning reduces infarct size and edema in patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol*. 2012;59:2175-2181.
18. Grande P, Hansen BF, Christiansen C, Naestoft J. Estimation of acute myocardial infarct size in man by serum CK-MB measurement. *Circulation*. 1982;65:756-764.
19. Gibson CM, Cannon CP, Daley WL, et al. TIMI frame count: A quantitative method of assessing coronary artery flow. *Circulation*. 1996;93:897-888.
20. Van' Hof AWJ, Liem A, Suryapranata H, Hoorntje JCA, Jan de Boer M, Zijlsta F. Angiographic assessment of myocardial reperfusion in patients treated with primary angioplasty for acute myocardial infarction: myocardial blush grade. *Circulation*. 1998;97:2302-2306.

21. Schröder R. Prognostic impact of early ST-segment resolution in acute ST-elevation myocardial infarction. *Circulation*. 2004;110:e506-e510.
22. Pocock SJ, Current controversies in data monitoring for clinical trials. *Clinical Trials*. 2006;3:513-521.
23. Schulz KF, Altman DG, Moher D; CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Ann Intern Med*. 2010 Jun 1;152(11):726-32.-481.
24. Rentoukas I, Giannopoulos G, Kaoukis A, et al. Cardioprotective role of remote ischemic periconditioning in primary percutaneous coronary interventions. *JACC Cardiovasc Interventions*. 2010;3:49-55.
25. Loukogeorgakis SP, William R, Panangiotidou AT, et. al. Transient Limb Ischemia Induces Remote Preconditioning and Remote Postconditioning in Humans by a KATP Channel–Dependent Mechanism. Circulation. 2007;116:1386-1395.
26. Garcia-Dorado D, Oliveras J, Gill J. Myocardial oedema: a preventable cause of reperfusion injury? *Cardiovasc Res*. 1993;27:1555-1563.
27. Friedrich MG. Myocardial edema, a new clinical entity? *Nat Rev Cardiol*. 2010;7:292-296.
28. de Lemos JA, Braunwald E. ST-segment resolution as a tool for assessing the efficacy of reperfusion therapy. *J Am Coll Cardiol*. 2001;30:1283-1294.

Figure titles and legends

Figure 1: Study design - Remote ischemic postconditioning. I = ischemia, R = reperfusion.

Figure 2: Study flowchart - Patients flow, STEMI = ST-elevation myocardial infarction, PCI = percutaneous coronary interventions, RIPC = remote ischemic postconditioning, ce-CMR = contrast-enhanced cardiac magnetic resonance. CABG = coronary artery bypass graft.

Figure 3: Primary endpoint - Area under the curve of CK-MB release, 72 hours after randomization. RIPC patients: 8814 arbitrary units (interquartile range, 5567 to 11325); controls: 10065 arbitrary units (interquartile range, 7465 to 14004) in controls; mean relative reduction 20%, 95%CI, 0.2% to 28.7%, P=0.043. Blue lines (pPCI + RIPC), red lines (pPCI), green bars (95% confidence interval).

Figure 4: Enzymatic infarct size and ce-CMR T2W edema - panel A. area under the curve of CK-MB release (log transformed) P=0.043; panel B. T2-weightened myocardial edema volume, P=0.049.

Table 1. Baseline characteristics and procedural data.

	pPCI + RIPC (n=48)	pPCI (n=48)
Age (years)	61 (11)	56 (11)
Men	41 (85)	43 (90)
Body mass index (Kg/m^2)	26 (4)	27 (4)
Family history of coronary artery disease	12 (26)	14 (30)
Hypertension	25 (54)	25 (53)
Active smokers	20 (53)	26 (54)
Dislipidemia	14 (30)	16 (33)
Diabetes mellitus	4 (9)	7 (15)
Previous myocardial infarction	6 (12)	5 (10)
Previous percutaneous coronary interventions	4 (8)	4 (8)
Pre-infarctual angina	16 (33)	14 (29)
Baseline heart rate (bpm)	75 (15)	81 (18)
Baseline serum creatinine (mg/dl)	0.96 (0.27)	1.01 (0.22)
Out of hospital cardiac arrest	3 (6)	4 (8)
Pain to balloon time (min)	178 [140-230]	183 [152-251]
Vessels with critical stenosis:		
1-vessel disease	28 (60)	32 (67)
2-vessel disease	13 (28)	9 (19)
3-vessel disease	6 (12)	7 (14)
LAD segment of occlusion:		
Proximal	24 (50)	21 (44)
Medium	20 (42)	26 (54)
Distal	4 (8)	1 (2)
Initial TIMI flow 1	5 (11)	1 (2)

Anti-GP IIb-IIIa	48 (100)	43 (91)
Thrombectomy	37 (77)	41 (85)
Stent		
Bare metal	30 (64)	32 (68)
Drug eluting	16 (34)	15 (32)
POBA	1 (2)	0 (0)
Contrast Volume (cc)	211 (55)	229 (72)
Morphine	26 (57)	25 (57)
Inotropes	2 (4)	4 (8)
Intra Aortic Balloon Pump	1 (2)	4 (8)

Categorical data are reported as number (proportion). Continuous variable are reported as mean (standard deviation) or median [interquartile range] as appropriate. RIPC = remote ischemic postconditioning, anti-GP IIb-IIIa = inhibitors of glycoprotein IIb-IIIa, POBA = plain only balloon angioplasty.

Table 2. Secondary clinical endpoint at 12 months.

	pPCI + RIPC (n=48)	pPCI (n=48)
Death	0	1
Stroke	0	1
Myocardial infarction	0	1
Repeated PCI (any vessel)	3	0
CABG	1	0

RIPC = remote ischemic postconditioning, PCI = percutaneous coronary interventions, CABG = coronary artery bypass grafting.

Table 3.Angiographical and ECG endpoints.

	pPCI + RIPC (n=48)	pPCI (n=48)	p-value
Final TIMI flow:			
0	0	0	0.554
1	1 (2)	2 (4)	
2	10 (21)	14 (29)	
3	37 (77)	32 (67)	
TIMI frame count (frames)	21 (8)	23 (9)	0.170
MBG:			0.067
Grade 0/1	5 (11)	13 (28)	
Grade 2/3	39 (89)	34 (72)	
STR_{50}	28 (66)	16 (37)	0.015
STR_{70}	12 (29)	4 (9)	0.049

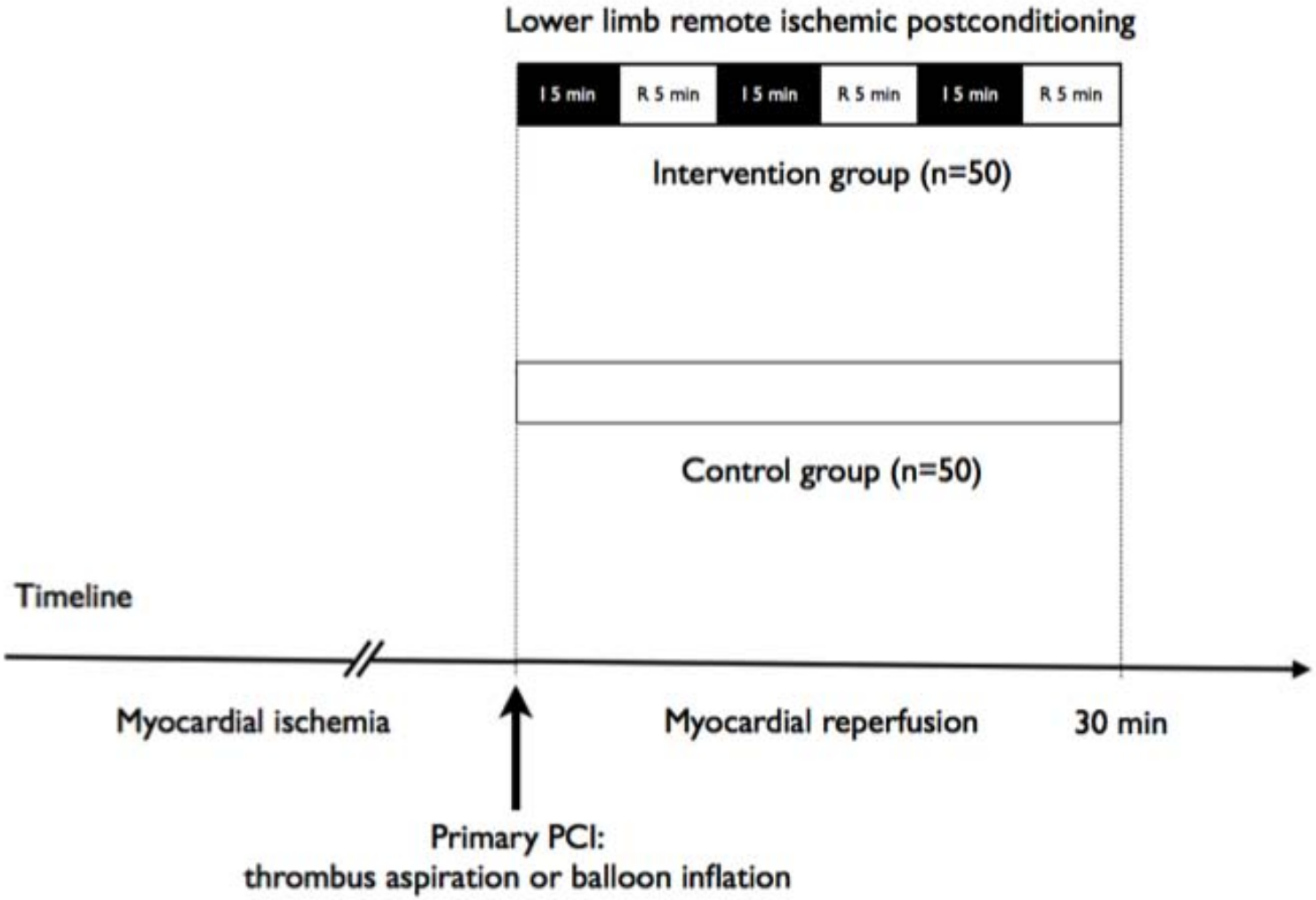
Categorical data are reported as number (proportion). Continuous variable are reported as mean. (standard deviation). RIPC = remote ischemic postconditioning, MBG = myocardial blush grading, STR = ST-segment resolution.

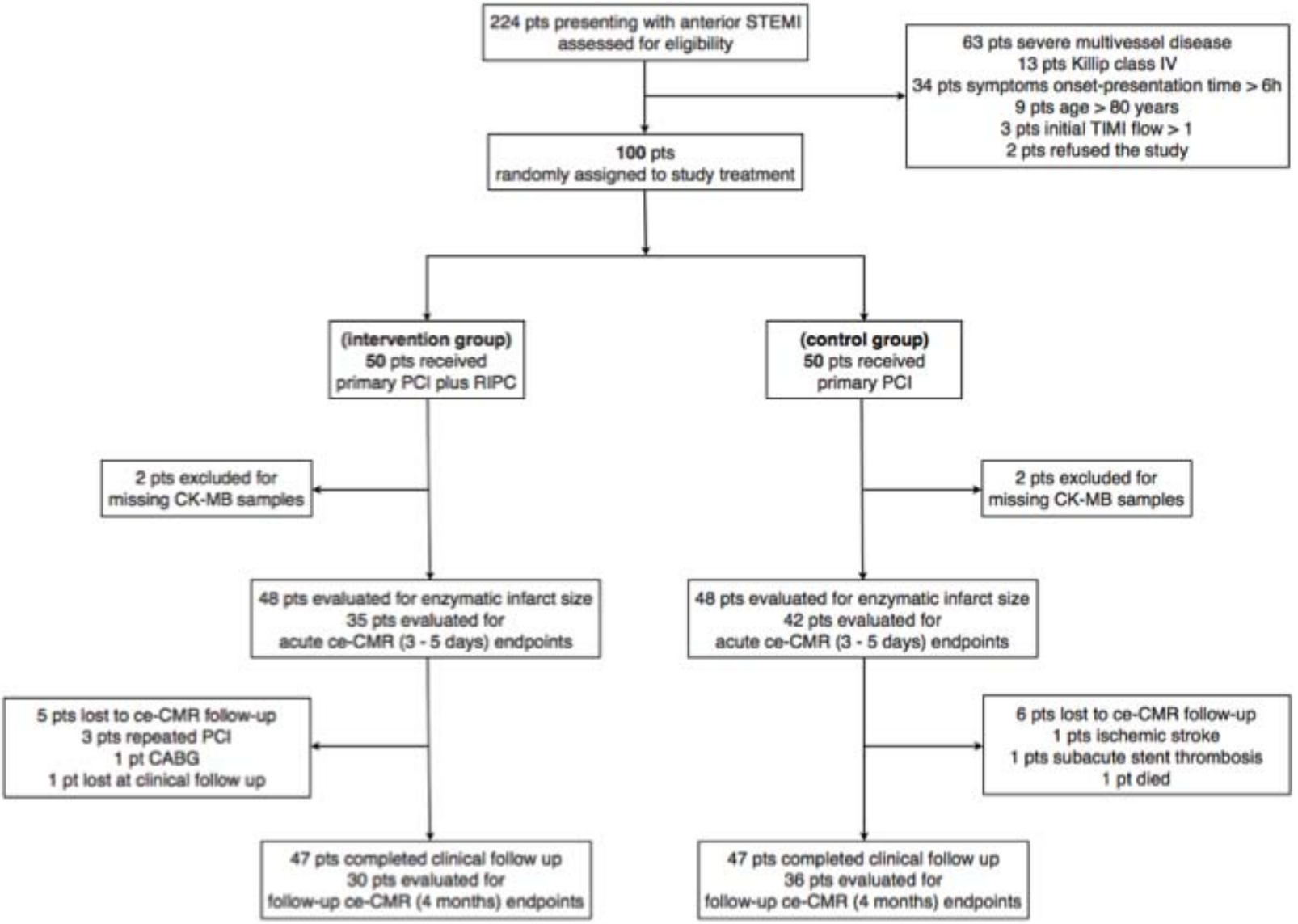
Table 4. Contrast-enhanced magnetic resonance findings, acute phase and 4 months follow-up.

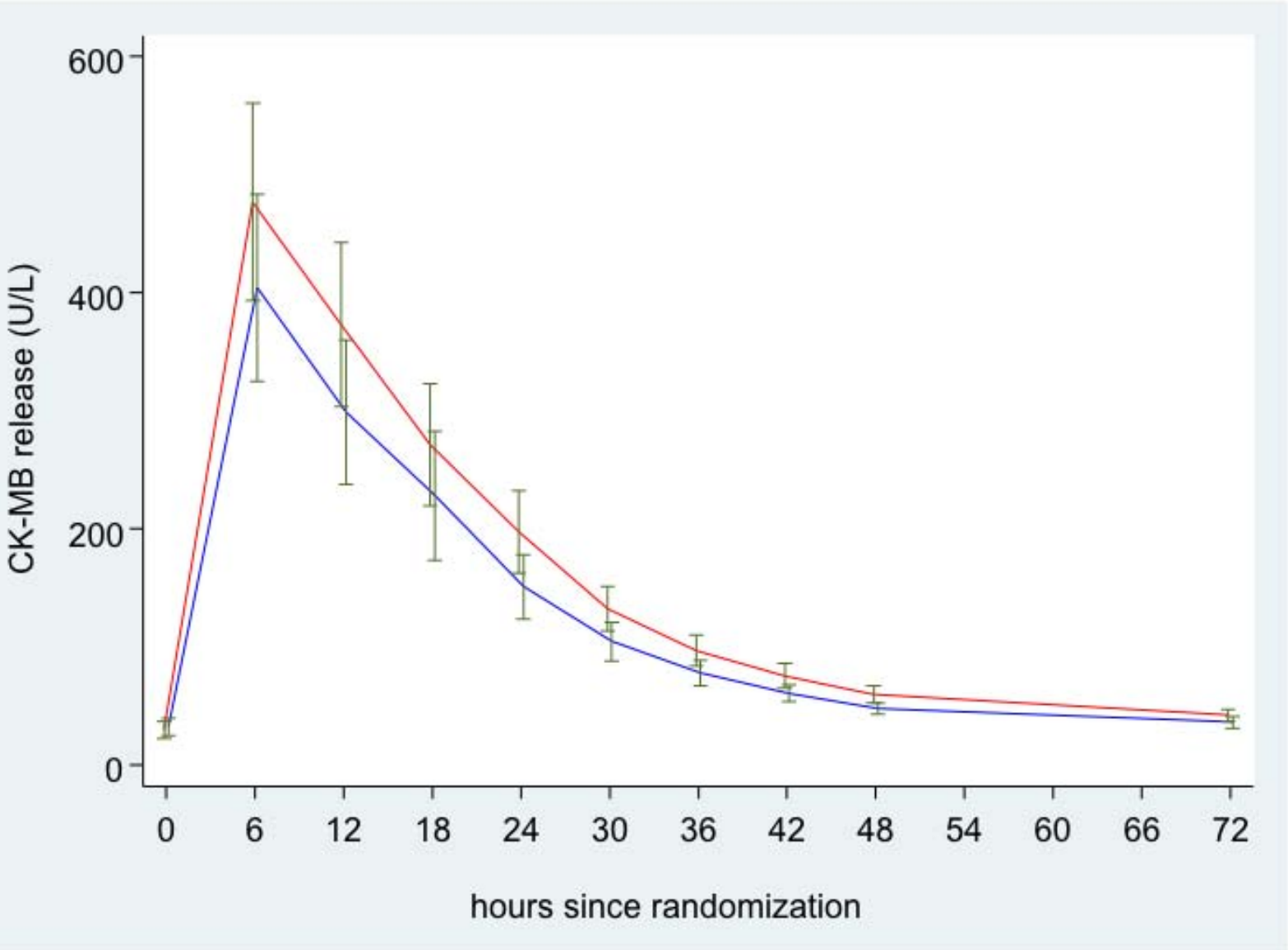
	Acute phase (3 – 5 days)			Follow-up (4 months)		
	pPCI + RIPC (n=35)	pPCI (n=42)	p-value	pPCI + RIPC (n=30)	pPCI (n=36)	p-value
Days to ce-CMR	4.7 (2.4)	5.1 (1.8)	0.135	351 [179-645]	319 [203-579]	0.823
LV end-diastolic volume (ml)	148 (37)	169 (52)	0.102	168 (45)	190 (73)	0.297
LV end-systolic volume (ml)	84 (26)	102 (46)	0.128	93 (39)	107 (60)	0.260
LV ejection fraction (%)	44 (9)	41 (9)	0.102	46 (10)	46 (11)	0.961
LV mass (gr)	111 (26)	125 (38)	0.098	103 (23)	118 (37)	0.158
Delayed enhancement (ml)	27 (14)	34 (19)	0.113	21 (12)	23 (13)	0.500
Delayed enhancement/LV mass ratio (%)	24 (10)	26 (13)	0.397	20 (10)	20 (9)	0.938
Edema (ml)	37 (16)	47 (22)	0.049			
Edema/LV mass ratio (%)	33 (11)	36 (15)	0.291			
Delayed enhancement/edema ratio (%)	72 (17)	72 (19)	0.987			
Presence of hemorrhage (%)	19 (68)	16 (53)	0.294			

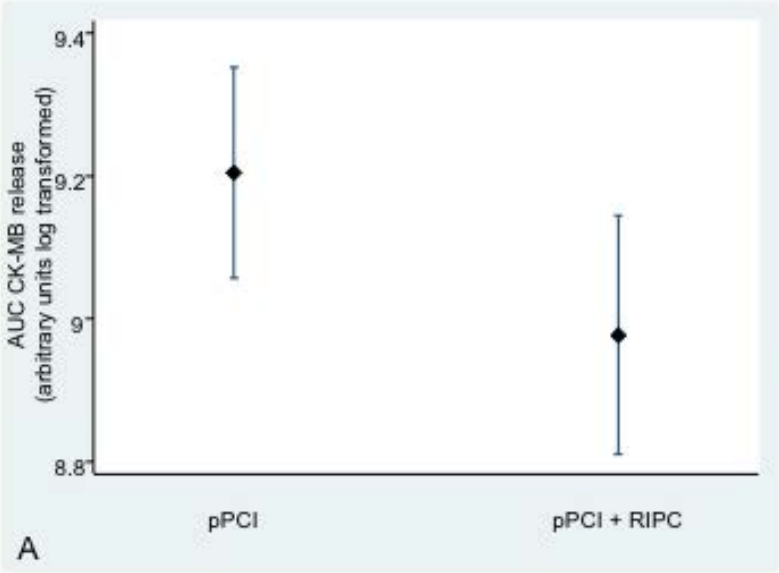
Presence of micro-vascular obstruction (%)	24 (77)	27 (75)	1.000

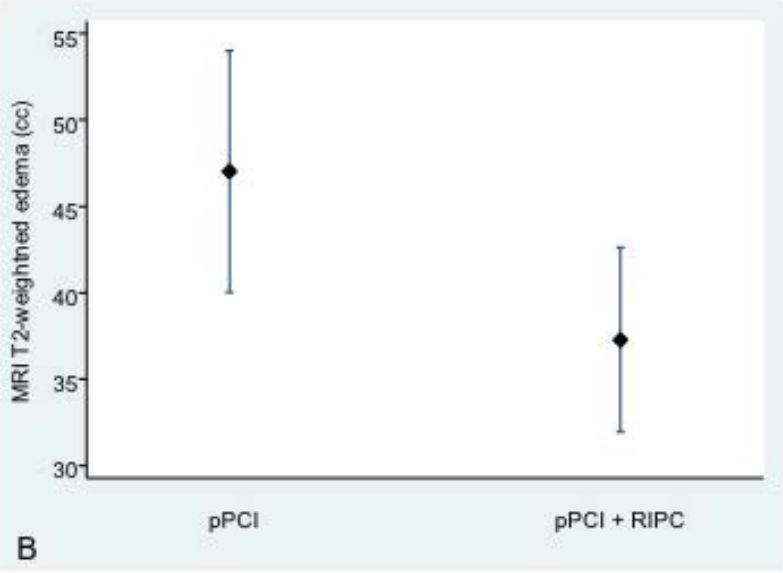
Categorical data are reported as number (proportion). Continuous variable are reported as mean (standard deviation) or median [interquartile range] as appropriate. RIPC = remote ischemic postconditioning, LV = left ventricle.


Lower limb remote ischemic postconditioning
I 5 min
R 5 min
I 5 min
R 5 min
I 5 min
R 5 min
Intervention group (n=50)
Control group (n=50)
Timeline
Myocardial ischemia
Myocardial reperfusion
30 min
Primary PCI:
thrombus aspiration or balloon inflation


224 pts presenting with anterior STEMI
assessed for eligibility
63 pts severe multivessel disease
13 pts Killip class IV
34 pts symptoms onset-presentation time > 6h
9 pts age > 80 years
3 pts initial TIMI flow > 1
2 pts refused the study
100 pts
randomly assigned to study treatment
(intervention group)
50 pts received
primary PCI plus RIPC
(control group)
50 pts received
primary PCI
2 pts excluded for
missing CK-MB samples
2 pts excluded for
missing CK-MB samples
48 pts evaluated for enzymatic infarct size
35 pts evaluated for
acute ce-CMR (3 - 5 days) endpoints
48 pts evaluated for enzymatic infarct size
42 pts evaluated for
acute ce-CMR (3 - 5 days) endpoints
5 pts lost to ce-CMR follow-up
3 pts repeated PCI
1 pt CABG
1 pt lost at clinical follow up
6 pts lost to ce-CMR follow-up
1 pts ischemic stroke
1 pts subacute stent thrombosis
1 pt died
47 pts completed clinical follow up
30 pts evaluated for
follow-up ce-CMR (4 months) endpoints
47 pts completed clinical follow up
36 pts evaluated for
follow-up ce-CMR (4 months) endpoints


600
400
200
0
CK-MB release (U/L)
0
6
12
18
24
30
36
42
48
54
60
66
72
hours since randomization


9.4
9.2
9
8.8
AUC CK-MB release
(arbitrary units log transformed)
pPCI
pPCI + RIPC
A


55
50
45
40
35
30
MRI T2-weightned edema (cc)
pPCI
pPCI + RIPC
B

Appendix

Supplementary figures

eFigure 1: Correlation between enzymatic infarct size and ce-CMR delayed gadolinium enhancement volume (3-5 days) - ce-CMR delayed enhancement volume is showed as a function of enzymatic infarct size (Area under the curve of CK-MB release). There was a significant correlation between the two modalities of infarct size evaluation ($R=0.70$, $P<0.001$).

eFigure 2: Correlation between acute T2-weightened edema and area of delayed gadolinium enhancement at cardiac magnetic resonance (follow-up 4 months) - ce-CMR delayed enhancement volume at follow-up volume is showed as a function of T2-weightned edema volume. We found a significant correlation between the two variables ($R=0.70$, $P<0001$).

Supplementary tables

eTable 1. Baseline therapy.

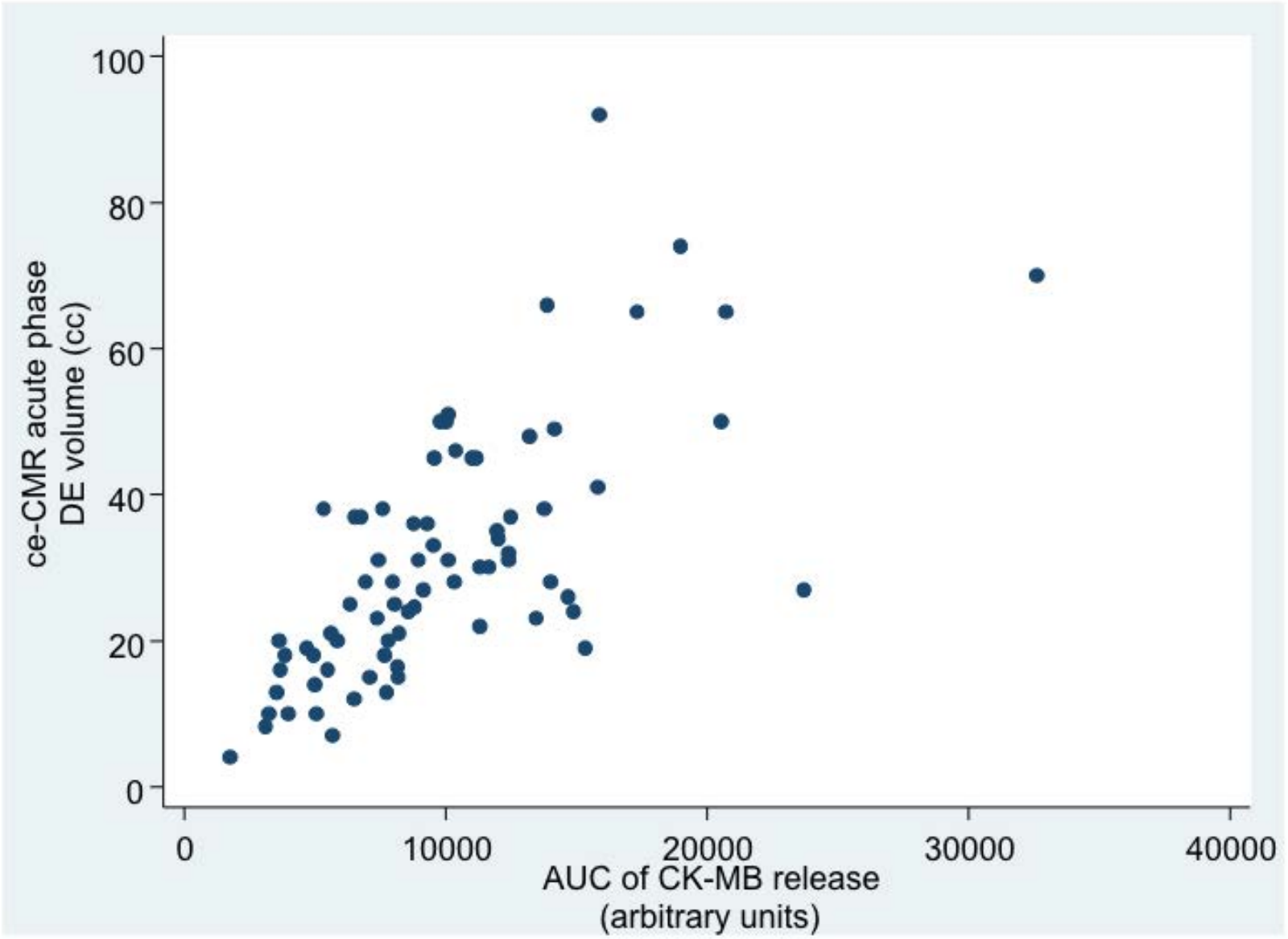
	pPCI + RIPC (n=48)	pPCI (n=48)
Baseline ACE inhibitors	4 (9)	5 (11)
Baseline ARB	3 (7)	2 (5)
Baseline beta blockers	6 (13)	9 (20)
Baseline diuretics	3 (7)	0 (0)
Baseline calcium channel blockers	3 (7)	4 (9)
Baseline statins	3 (7)	1 (2)

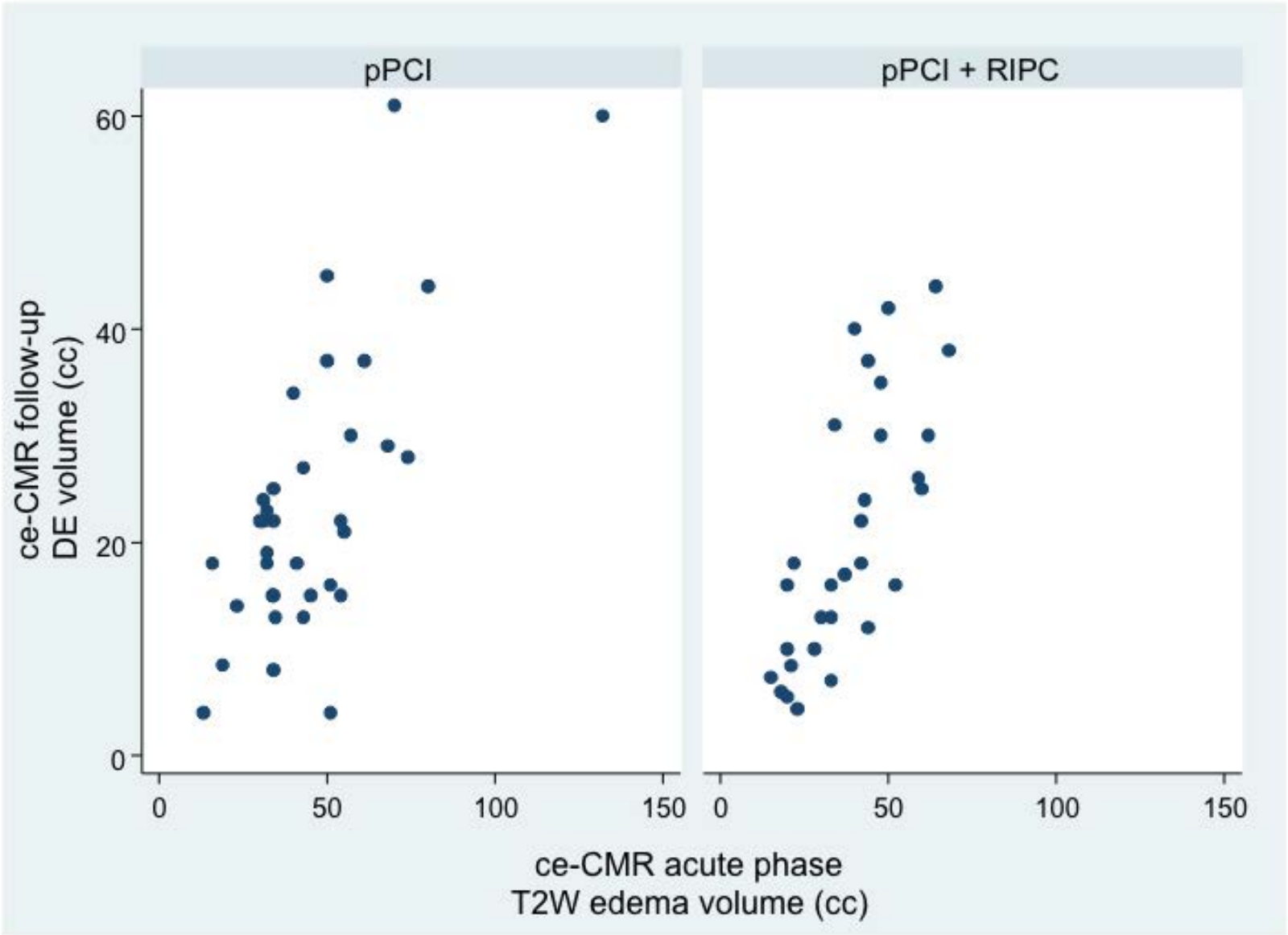
ACE = angiotensin converting enzymes, ARB = angiotensin receptor blockers.

eTable 2. Contrast – enhanced magnetic resonance endpoints, acute phase and follow-up: right ventricle findings.

	Acute phase (3-5 days)			Follow-up (4 months)		
	pPCI +RIPC (n=35)	pPCI (n=42)	p-value	pPCI + RIPC (n=30)	pPCI (n=36)	p-value
LV end-diastolic volume indexed (ml/mq)	85 (19)	90 (24)	0.368	96 (24)	101 (31)	0.485
LV end-systolic volume indexed (ml/mq)	48 (14)	55 (23)	0.331	53 (21)	57 (27)	0.485
LV mass indexed (gr/mq)	64 (13)	66 (16)	0.710	59 (9)	62 (13)	0.330
RV end-diastolic volume (ml)	97 (29)	98 (27)	0.857	110 (24)	118 (36)	0.318
RV end-diastolic volume indexed (ml/mq)	54 (17)	52 (11)	0.641	64 (13)	62 (11)	0.727
RV end-systolic volume (ml)	36 (15)	35 (14)	0.685	39 (13)	43 (17)	0.353
RV end-systolic volume indexed (ml/mq)	21 (8)	19 (6)	0.225	22 (7)	22 (6)	0.892
RV ejection fraction (%)	65 (9)	65 (6)	0.913	65 (6)	66 (7)	0.927

Categorical data are reported as number (proportion). Continuous variable are reported as mean (standard deviation) or median [interquartile range] as appropriate. RIPC = remote ischemic postconditioning.


ce-CMR acute phase
DE volume (cc)
100
80
60
40
20
0
0
10000
20000
30000
40000
AUC of CK-MB release
(arbitrary units)


pPCI
pPCI + RIPC
ce-CMR follow-up
DE volume (cc)
0
20
40
60
0
50
100
150
ce-CMR acute phase
T2W edema volume (cc)