Effect of COMBinAtion Therapy with remote ischemic conditioning and exenatide on the Myocardial Infarct size: a two-by-two factorial randomized trial (COMBAT-MI)

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† In the memory of Professor Garcia-Dorado, who passed away on August 16, 2019.

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ABSTRACT.

Remote Ischemic Conditioning (RIC) and the GLP-1 analog exenatide activate different cardioprotective pathways and may have additive effects on infarct size (IS). Here, we aimed to assess the efficacy of RIC as compared with sham procedure, and of exenatide, as compared with placebo, and the interaction between both, to reduce IS in humans. We designed a two-by-two factorial, randomized controlled, blinded, multicenter, clinical trial. Patients with ST-segment-elevation myocardial infarction receiving primary percutaneous coronary intervention (PPCI) within 6 hours of symptoms were randomized to RIC or sham procedure and exenatide or matching placebo. The primary outcome was IS measured by late gadolinium enhancement in cardiac magnetic resonance performed 3-7 days after PPCI. The secondary outcomes were myocardial salvage index, transmurality index, left ventricular ejection fraction and relative microvascular obstruction volume. A total of 378 patients were randomly allocated and, after applying exclusion criteria, 222 patients were available for analysis. There were no significant interactions between the two randomization factors on the primary or secondary outcomes. IS was similar between groups for the RIC (24±11.8% in the RIC group vs 23.7±10.9% in the sham group, p=0.827) and the exenatide hypotheses (25.1±11.5% in the exenatide group vs 22.5±10.9% in the placebo group, p=0.092). There were no effects with either RIC or exenatide on the secondary outcomes. Unexpected adverse events or side effects of RIC and exenatide were not observed. In conclusion, neither RIC nor exenatide, or its combination, were able to reduce IS in STEMI patients when administered as an adjunct to PPCI.

Keywords: Remote ischemic conditioning, exenatide, ST-segment-elevation acute myocardial infarction, primary percutaneous coronary intervention.

INTRODUCTION

Prompt reperfusion with primary percutaneous coronary intervention (PPCI) in patients with ST-segment elevation myocardial infarction (STEMI) is the most effective therapy for limiting myocardial infarct size (IS). However, there are ~~some shortcomings in the~~ daily clinical practice. On the one hand, the time window for effective IS reduction is narrow [14]. On the other hand, reperfusion itself can induce further myocardial injury accounting up to 50% of the final IS [49], a phenomenon known as ischemia/reperfusion injury (IRI) [33].

Among the many approaches that have been proposed to limit IRI in patients with STEMI, remote ischemic conditioning (RIC) and the administration of glucagon-like peptide-1 (GLP-1) analogs appeared especially attractive [15]. RIC has been considered until very recently a promising strategy, since in many clinical studies conducted in STEMI patients it increased myocardial salvage and reduced IS by 20–30% when applied before or during reperfusion [5,7,10,34,35,45,48]. However, the cardioprotective effect of RIC is currently under debate. In a recently published randomized clinical trial, RIC had no effect on clinical outcomes or on IS evaluated by cardiac biomarkers [18]. This discrepancy has been attributed to the use of different RIC protocols or to a potential type I error in those trials that showed cardioprotective effects [18].

GLP-1 is an incretin hormone that decreases blood glucose by stimulating insulin production and secretion in response to nutrient intake. The GLP-1 receptor promotes glucose uptake by myocardial cells improving their metabolic efficiency [44,50], and activates different cardioprotective biochemical pathways against IRI [3,31,37]. GLP-1 analogs, such as exenatide and liraglutide, are currently used to reduce blood glucose in type 2 diabetic patients, and ~~it has been reported their usefulness for reducing~~ IS in preclinical animal models of acute IRI [41,43] and in proof-of-concept clinical trials [6,29,46].

Emerging data suggest that reduction of IRI may require the additive or synergistic effects of multitarget strategies [8]. A study conducted by our group showed that exenatide and RIC activate different cardioprotective pathways and had additive effects on IS reduction after transient coronary occlusion in pigs [24]. However, it is unknown

whether this combined effect of RIC and exenatide also occurs in patients when administered during the acute phase of STEMI. For this reason, we conducted the COMBAT-MI trial (a two-by-two factorial, randomized controlled, multicenter, clinical trial) to assess the efficacy of RIC as compared with a sham procedure, and the GLP-1 analog exenatide, as compared with placebo, and the combined effect of both strategies to reduce IS assessed by cardiac magnetic resonance (CMR) in patients with STEMI receiving PPCI.

METHODS

The COMBinAtion Therapy in Myocardial Infarction (COMBAT-MI, registered at www.clinicaltrials.gov (NCT02404376); EudraCT number, 2015-001000-58.) was an investigator-initiated, prospective, randomized, multicenter, two-by-two factorial, double blinded, clinical trial, comparing RIC with a sham procedure and exenatide with matching placebo in patients with STEMI undergoing PPCI. The study was conducted in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments and the European guidelines for Good Clinical Practice, and was approved by the *Agencia Española de Medicamentos y Productos Sanitarios* (AEMPS) and the Ethics Committees of the participant institutions. Data from 6 tertiary academic centers in Spain were included. All participants provided written informed consent before randomization.

Patient selection and randomization.

Patients eligible for enrolment were ≥18 years old with a diagnosis of STEMI characterized by chest pain or other ischemic symptoms and ≥1 mm ST elevation in ≥2 leads in the same territory or ≥2 mm ST elevation in ≥2 V1 through V4 leads or left bundle branch block with ≥1 mm concordant ST elevation, and presenting within 6 hours of symptom onset.

Exclusion criteria were known hypersensitivity to exenatide or any of excipients, contraindication to CMR, assumed life expectancy below one year, pregnancy, ongoing participation in another trial, clinical instability that led to inability to study comprehension (i.e. loss of consciousness, confusion, profound cardiogenic shock) and TIMI flow grade >1 at the time of diagnostic coronary angiography.

Allocation to treatments in each participating center was done via a web-based clinical trial support system accessible 24h a day (W3NEXUS, Barcelona Spain). Stratification was internally determined by each participating center using permuted blocks with sizes of 4, 6 or 8. Patients eligible were enrolled in the emergency room or upon entering the catheterization laboratory and were randomly assigned to one of four groups according to 1:1:1:1 ratio. Table 1 shows patient distribution in all four groups.

Study procedures.

In the RIC procedure, an automated cuff (autoRIC™, CellAegis Devices, partner CELL; Toronto; Canada) was placed on the upper arm and inflated to 200mmHg for 5 min and then deflated for 5 min, a cycle which was programmed to be undertaken 4 times in total, as previously described [5]. The RIC protocol was applied trying to start at least 20 min before artery aperture (balloon inflation or stent deployment) and all patients completed the 4 cycles. For the control group, a sham cuff was placed on the upper arm that simulated the autoRIC™ device with the same sound and vibration. The total number of inflations at which coronary blood flow was restored was registered.

In the exenatide arm, an intravenous infusion of exenatide (18μg) diluted in saline (vehicle, 180mL) was intravenously administered, prior to the PPCI, at a flow rate of 72mL/h (0.12μg/min). After 15 min, the flow rate was reduced to 26mL/h (0.043μg/min) and maintained for additional 6 hours. This protocol was previously found to be safe and effective against IRI in STEMI patients [28]. In the matching-placebo arm, vehicle was administered following the same protocol used for exenatide infusion. Exenatide or vehicle infusions were started following randomization and before obtaining arterial access for cardiac catheterization, with no delay to application of reperfusion therapy. Only ~~a~~ research nurse was unblinded and prepared the treatment assigned according to the randomization process in the cath lab. The investigator team collecting and analyzing the data ~~was~~ blinded to treatment allocation.

Cardiac catheterization was performed by experienced operators in PPCI without any delay ~~due to the acceptance to participate in the trial~~, and stenting was performed according to the usual procedures. The PPCI procedure followed guideline recommendations [25], according to local practice. Thrombectomy and selection of antiplatelet and anticoagulant regimens were per operator discretion.

CMR Protocol.

All CMR studies were performed in a Siemens or Philips 1.5 T or 3 T clinical scanner using a phased-array cardiac receiver coil at 3-7 days following the PPCI procedure and a standarized CMR image protocol. Electrocardiogram-gated breath-hold short-axis cine views were performed to quantify volumes and ejection fraction (SSFP sequences; slice thickness: 6 mm; space between slices 67%; matrix: 256x256: field of view: 300-370mm; temporal resolution <50ms). Additional 2-chamber, 3-chamber and 4-chamber views were also obtained. ~~LGE~~ images were acquired at identical slice positions to the cine images after the administration of 0.2 mmol/kg of body weight Gadolinium-DTPA (Gd-DTPA) (Berlex, Montville, NJ, USA).

STIR sequences were used in the same view as the cine sequences, all in mid-diastole to evaluate ~~the~~ edema, (slice thickness: 8mm; space between slices 20%; matrix: 256x256: FOV: 300-370mm; temporal resolution <50ms; repetition time: 2 R-R intervals; echo time: 100 ms; inversion time: 170 ms; flip angle: 160°; bandwidth, 781 Hz/pixel).

A segmented inversion-recovery (seg-IR) gradient-echo sequence was acquired starting at 25 min after contrast administration to minimize IS overestimation associated to myocardial edema as previously described [36] (Matrix 256 x 197, voxel size 2.0 x 1.6 x 6 mm, TE 4.91 ms, TR 700 ms, flip angle 30º; and the bandwidth 140 z/pixel).

The images were centrally analyzed by experts blinded to group allocation. Image analysis was conducted on a workstation (Cvi42, Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada) by 2 cardiologists specialized in cardiac imaging. Data on reproducibility, inter- and intraobserver variability have been previously published [32].

Study endpoints.

The primary study endpoint was myocardial IS measured by late gadolinium enhancement (LGE) in a CMR performed 3-7 days after PPCI and expressed as a percent of the left ventricular (LV) mass.

Secondary study endpoints included: 1) myocardial salvage index (MSI), defined as ratio of LGE to the extent of myocardial edema, assessed by T2-weighted CMR; 2) the

transmurality index, defined as the ratio of the mass of myocardium showing LGE to the mass of the myocardial segment containing it; 3) the left ventricular end-diastolic volume (LVEDV) and left ventricular ejection fraction (LVEF), as determined by CMR imaging; and 4) the relative microvascular obstruction volume (MVO) compared to IS measured on LGE sequences of IS.

Sample size calculation and statistical analysis.

The trial used a factorial design to evaluate two hypotheses. One hypothesis was that RIC would be superior to a sham procedure with regard to the outcome IS. The second hypothesis was that exenatide would be superior to matching placebo with regard to the same outcome. If both hypotheses were true, a third hypothesis of an interaction effect between RIC and exenatide would be tested.

The population for the primary and secondary outcome analyses included all patients who underwent randomization, received at least one cycle of RIC intervention (or sham procedure) at the time of myocardial reperfusion, had a baseline TIMI ≤ 1, underwent CMR within the specified timeframe and the analysis of the primary outcome in the CMR was available.

To calculate sample size, we assume a conservatively IS of 24% with a standard deviation of 14 based on previous studies. Sample size was computed to detect a reduction of IS of at least 20% with either RIC or exenatide or with the combined therapy, with a statistical power $1-\beta= 0.8$ and a significance level $\alpha = 0.05$. This resulted in a calculated total sample size of 274 patients. Assuming that diagnostic coronary angiography could not confirm the initial diagnosis of STEMI in 2% of patients receiving the study treatment, and that in an additional 20% TIMI flow would be >1, a total of 351 should be included to ensure the calculated sample size. This figure was increased to 378 to allow a loss of patients with confirmed STEMI and TIMI ≤ 1 not completing an adequate CMR study.

Continuous variables were described using mean and standard deviation for normally distributed variables and median/interquartile ranges for non-normal variables. Categorical variables were described using absolute and relative frequencies. Baseline differences between groups were compared using t-Student test or exact Fisher test

where appropriate. The effect of exenatide and RIC were assessed using a linear model. Interaction between the two randomization factors (RIC and exenatide) on the primary and secondary outcomes was assessed. If there was no significant interaction, the two factors were analyzed independently. In a first step the linear model incorporated the main effect for each treatment and the interaction term for exenatide and RIC. In case of no statistical effect of the interaction, the model was readjusted including the main effects of exenatide and RIC.

Pre-specified subgroup analyses were defined based on age (< 65 years vs ≥ 65 years), the infarct-related coronary artery (left anterior descending vs other), and time from symptom onset to reperfusion (< 120 min vs ≥ 120 min). P values were calculated for interaction tests of prespecified subgroups. The statistical analysis was performed using RStudio.

RESULTS.

From Mar-2016 through Jun-2019 a total of 378 patients were randomized from six sites to receive RIC or a sham procedure and to receive exenatide or matching placebo. The exclusion of patients for several reasons during PPCI and hospitalization led to a final sample of 222 patients randomized to the different arms available for the analysis (Figure 1).

The characteristics of the patients at baseline were well balanced between the trial groups (Table 2). The median age among all the patients was 60.5 years, and 16.2% were women. The prevalence of cardiovascular risk factors (hypertension, dyslipidemia, diabetes mellitus, peripheral arterial disease, chronic renal impairment, chronic obstructive pulmonary disease, as well as a clinical history of MI and stroke) was similar in the study groups. There were more patients randomized to placebo who were active smokers compared to the group randomized to exenatide (*P*= 0.023). There were not relevant differences in the baseline drugs treatment.

Table 3 shows procedure details. Most patients were in a stable hemodynamic condition and on Killip-I (n=186; 86.1%), without differences among groups. There were also no differences in the delay from the initial symptoms to arrival at the catherization lab (median 137 min) or in the mean hemoglobin and creatinine values on admission. The

most common infarct-related coronary artery was the right coronary artery (n=101; 45.7%), followed by the left anterior descending coronary artery (n=80; 36.2%) and, in most instances, the initial coronary TIMI flow was 0 (n=195, 88.2%). Coronary TIMI flow grade 3 was reached in 91.4% of cases. Most patients had received 2 (n=92; 41.8%) or 3 (n=73; 33.2%) RIC cycles at the time of coronary flow restoration, without differences among groups. There were no differences between groups in the use of aspiration thrombectomy or in the medications administered during the procedure.

CMR was performed 3-7 days after PPCI (median 5 days). Table 4 shows the results for the main hypotheses on the primary and secondary outcomes. There were no significant interactions between the two randomization factors on the primary or secondary outcomes, which together with the lack of significant effect for each treatment, implies no significant effect of the combined therapy. IS was similar between groups for the RIC hypothesis (24±11.8% in the RIC group vs 23.7±10.9% in the sham procedure group, *P*= 0.827), and for the exenatide hypothesis (25.1±11.5% in the exenatide group vs 22.5±10.9% in the placebo group, *P*= 0.092). Furthermore, there were no differences among groups in both hypotheses in LVEDV, either considering absolute or indexed values. Accordingly, LVEF was virtually identical between groups for the RIC hypothesis (45.2±10.1% in the RIC group vs 45.9±10.4% in the sham procedure, *P*= 0.60) and for the exenatide hypothesis (45.4±10.2% in the exenatide group vs 45.7±10.4% in the placebo group, *P*= 0.858). There were no differences between groups for the RIC hypothesis in MSI but, in the case of the exenatide hypothesis, those patients randomized to exenatide had a lower MSI than those randomized to placebo (22.3±15.8% vs 27.9±18.1%, *P*= 0.018), indicating an opposite direction of the hypothesized effect. Finally, there were no differences among groups, in both hypotheses, in the relative MVO and transmurality index.

In the pre-specified subgroup analyses based on age, the infarct-related coronary artery, and time from symptom onset to reperfusion, we did not detect any effect of RIC or exenatide on IS (Figure 2).

Exploratory clinical adverse events during hospitalization were similar in all groups in the intention-to-treat analysis (Table 5) and also in the safety analysis performed on the entire randomized sample (Table 6).

DISCUSSION

This two-by-two factorial, double blinded, randomized controlled, multicenter clinical trial was designed on the hypothesis of finding a benefit from RIC and exenatide, a GLP-1 analogue, to limit the size of the myocardial infarct. On this basis, we hypothesized a possible additional benefit of the combination of both therapies when administered adjunct to PPCI. After randomizing 378 patients to the four potential strategies, no effect of RIC or exenatide was found to reduce IS in patients with STEMI submitted to PPCI.

Our factorial design approach has been based on the positive results of some previous trials that tested each separate therapy [5,7,25,35,41] and on the positive results obtained in pigs by our group in 2015 [1]. Our results suggested a protective effect of RIC and exenatide and, most importantly, supported the superiority of combination therapy over the individual treatments to reduce IS. This additive effect was based on the fact that RIC and exenatide exerted protection by different mechanisms [1]. In the case of RIC, experimental studies have demonstrated that the cardioprotective signal is transferred from the remote conditioned organ or tissue, either through the release of humoral factors or through activation of neuronal pathways, to the heart, where it triggers cardioprotective signaling cascades [19]. In our preclinical study, RIC was associated with a reduction of nitro-oxidative stress in a pig model of transient coronary occlusion. Protection by GLP-1 analogs has been associated with modulation of glycolytic metabolism [23], a fact that was confirmed in our previous study using ^{1}H-NMR [1].

The results of our experimental study could not be replicated in the clinical setting. Many reasons, extensively reviewed elsewhere [4,8,20,21,26], have been proposed to explain the failure to translate experimental results to patients' cardioprotection. Also, some conceptual and technical errors in the design of clinical trials have contributed to this translation failure [4,16,20,21]. For instance, most preclinical research on both RIC and GLP-1 analogs has been carried out in young and healthy animals, in the absence of medications, commonly used in STEMI patients and that can modify the efficacy of both cardioprotective strategies [4,17,26,27]. Particularly important are the P2Y12 antagonists, which are nowadays widely used as routine anti-platelet therapy and induce

protection *per se* [2,47]. In the present study we have followed the guidelines for rigor and reproducibility of clinical studies as published by Botker et al. [4]. According to these authors, the gold standard for clinical trial design is a prospective, randomized, blinded, controlled study, with infarct size measured by CMR as the ideal primary end-point [4], characteristics that have been fulfilled in this work.

In the specific case of RIC, we applied the same protocol that was used in a proof-of-concept trial, with the difference ~~that in such study,~~ RIC was started in the ambulance [5] whereas we initiated RIC ~~at~~ hospital arrival. Thus, the number of complete RIC cycles before coronary flow restoration in that trial was four, whereas in our study only 17.6% of patients completed the entire protocol at the time of reperfusion, which may explain in part the lack of benefit of RIC as compared with the proof-of-concept trial. However, comparisons with that trial should be made cautiously, since the assessment of the MSI was done at 30 days of reperfusion and it was estimated by gated single photon emission tomography. In addition, prior research has shown that physiologic biochemical responses appear right after the first inflation [9].

Regarding exenatide, our protocol was based on that described by Lønborg et al [29], aimed to attain plasma concentrations of the GLP-1 analogue in a range previously shown to be beneficial (0.03 to 0.30 nmol/L) [38]. We have not quantified plasma concentration of exenatide in the present study, although it seems reasonable to think that it should be within the same range ~~that in~~ Lønborg's study, as the protocol was the same. Nevertheless, the possibility that the therapeutic level was not optimal cannot be excluded.

A major concern that could be related ~~with~~ the different results between studies is the lack of uniformity in the method used to quantify IS. In some studies it has been based on repolarization changes during STEMI (ST-segment deviation scores) or on the pattern of serum cardiac biomarkers release as surrogate markers [18,34,35,48]. These were unblinded studies, which could have led to some bias. Even considering only those studies that used imaging techniques to assess different strategies to decrease IRI, heterogeneity is also present. Four studies have shown benefit of RIC to reduce IS by imaging. However, they were relatively small size studies, their RIC protocols differed and, most importantly, they considered different endpoints [5,7,10,45]. In line with a

recent consensus document [24], we have used CMR-based IS (extent of LGE) as the primary outcome measure. In our study, the ratio of LGE to edema was included as a surrogate for myocardial salvage index. Data arising after the design of the trial demonstrated that edema is not an accurate surrogate for area at risk [11], especially when cardioprotective therapies are applied [12]. Indeed, CMR-based myocardial salvage index is no longer recommended in trials assessing the effect of cardioprotective therapies [42]. In any case, as we found no differences in the LGE to edema ratio, the selection of this secondary outcome had no impact on the trial results.

Different mechanisms depending on the method used to assess IS with CMR could justify the divergences among these studies. To start with, methods for IS quantification have differed, and have included manual planimetry [7], Otsu method [24,45], full width at half maximum [40], or standard deviations [10] as it was in our case. Another key point is the time elapsed from the contrast administration to the LGE sequence acquisition. Some studies have shown that this time interval is crucial in the acute phase due to the presence of myocardial edema, and that IS estimation can vary up to 20% depending on this time-interval [30,36]. In our study protocol, LGE sequences were acquired 25 minutes after contrast administration to minimize this effect. Other studies with a similar methodology [10,24] did not show any differences in IS post-RIC when the acquisition was delayed up to 15-20 minutes post-contrast. In the same manner, the absence of edema reduction in our study, as compared with other studies [7,40,45], could also reflect differences in the time of performing CMR after PPCI, since edematous reaction is bimodal [13].

After the results of ~~Hausenloy's study~~ [18], which was an appropriately powered trial, a potential impact of RIC alone on short or medium term clinically relevant outcomes seems unlikely. However, it has been reported that the combination of intrahospital RIC and ischemic postconditioning increases myocardial salvage assessed by CMR compared to conventional PPCI [10] and reduces the rate of major adverse cardiac events and heart failure after long-term follow-up [39]. These results suggest that in addition to the excellent results achieved with contemporary treatment of STEMI, the implementation of optimized conditioning protocols may further improve clinical outcome of STEMI patients. It has been proposed that future trials on cardioprotection should focus on patients who really need adjunctive therapies, including those with

Killip class ≥3, where there is still room for improved clinical outcomes, as well as on patients with limited access to modern reperfusion therapies (i.e., developing countries) [21,22].

The present study has several limitations. First of all, the number of patients with >1 TIMI flow at the initial coronary angiogram was unexpectedly high. Therefore, the total number of patients available for the primary analysis was 222, whereas the calculated total sample size was 274 patients. A post-hoc analysis based on the results obtained with 222 patients revealed as unlikely a type II error (1.1% for the exenatide hypothesis, 7.1% for the RIC hypothesis and 0.1% for the interaction hypothesis). As early mentioned, a significant proportion of patients (41.8%) had only completed 2 cycles of the autoRIC protocol at the time of coronary flow restoration, which could limit the efficacy of the protection. We did a post-hoc subgroup analysis considering only those patients who had received at least 3 cycles at the moment of coronary flow restoration (n=117), and no benefit was observed either (data not shown). Finally, we decided not to include the pattern of cardiac biomarkers release as an endpoint because it is only a surrogated outcome of the actual IS and would require uniform measurement in a central laboratory to achieve good accuracy, which was not possible in this study. In any case, in a subanalysis of 146 patients with valid data from the coordinator center, there were also no differences between the groups in the peak median values of CK-MB neither in the RIC hypothesis (median 261.85, Interquartile range [141.85 - 337.15] in the RIC group and 234.2 [174 - 353.6] in the sham group; p=0.43) nor in the exenatide hypothesis (266.1 [174 - 345.4] in the exenatide group and 229.95 [145.6 - 330.9] in the placebo group; p=0.33).

In conclusion, the results of our trial suggest no benefit on IS reduction in the short term when RIC, exenatide or both therapies combined are administered as an adjunct to PPCI. Whether a medium or long-term benefit on myocardial function and remodeling could be observed in some patients should be explored in future clinical trials.

DECLARATIONS:

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Conflicts of interest Authors declare that they have no conflict of interest.

Ethics approval This study was conducted in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments and the European guidelines for Good Clinical Practice and was approved by the *Agencia Española de Medicamentos y Productos Sanitarios* (AEMPS) and the Ethics Committees of the participant institutions.

Consent to participate All participants provided written informed consent before randomization.

Consent for publication The manuscript is not submitted elsewhere nor is under consideration for publication. All authors have read and agreed with the submission of the manuscript.

Availability of data and material All data and materials used for this study are displayed or can be displayed upon request.

FIGURE LEGENDS:

Figure 1. Study flow chart. (A): Remote ischemic conditioning (RIC) vs sham remote ischemic conditioning. (B) Exenatide vs matching placebo. Abbreviations: CMR: Cardiac magnetic resonance; PPCI: Primary percutaneous coronary intervention; STE: ST-segment elevation.

Figure 2. Forest plot representing the estimated effect on the relative necrotic mass (%) for pre-specified subgroup analyses of the primary endpoint in the intention-to-treat population. Abbreviations: LAD= Left anterior descending.

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Table 1. Number of patients in the four treatment groups.

	Sham RIC	**RIC**
Matching placebo	58	54
Exenatide	62	48

Table 2: Baseline characteristics of the patients.

	RIC	Sham RIC	*P* Value	Exenatide	Matching Placebo	*P* Value	Total
Number of patients, no.	102	120		110	112		222
Age, yr, (mean ± SD)	62.2±10.9	60.8±11.6	0.339	61.7±11.5	61.2±11	0.771	61.5±11.3
Female, no. (%)	14 (13.7)	22 (18.3)	0.368	22 (20)	14 (12.5)	0.148	36 (16.2)
BMI, kg/m^2, (mean ± SD)	27.2±3.9	27.5±4	0.652	27.6±3.9	27.1±4	0.390	27.4±3.9
Active Smoker, no. (%)	48 (47.1)	54 (45)	0.788	42 (38.2)	60 (53.6)	0.023	102 (45.9)
Hypertension, no. (%)	46 (45.1)	57 (47.5)	0.787	45 (40.9)	58 (51.8)	0.109	103 (46.4)
Dyslipidemia, no. (%)	58 (56.9)	69 (57.5)	1.000	60 (54.5)	67 (59.8)	0.498	127 (57.2)
Medically treated diabetes, no. (%)	24 (23.5)	23 (19.2)	0.510	22 (20)	25 (22.3)	0.743	47 (21.2)
Peripheral arterial disease, no. (%)	9 (8.8)	6 (5)	0.292	10 (9.1)	5 (4.5)	0.191	15 (6.8)
Renal impairment (Cl <50), no. (%)	5 (4.9)	3 (2.5)	0.475	2 (1.8)	6 (5.4)	0.280	8 (3.6)
COPD, no. (%)	6 (5.9)	7 (5.8)	1.000	5 (4.5)	8 (7.1)	0.569	13 (5.9)
Previous AMI, no. (%)	1 (1)	3 (2.5)	0.627	0 (0)	4 (3.6)	0.122	4 (1.8)
Previous stroke, no. (%)	2 (2)	1 (0.8)	0.595	2 (1.8)	1 (0.9)	0.620	3 (1.4)
Medications at admission for PPCI, no. (%)							
Statins	27 (26.5)	26 (21.7)	0.432	25 (22.7)	28 (25)	0.754	53 (23.9)
Antiplatelet therapy	11 (10.8)	13 (10.8)	1.000	11 (10)	13 (11.6)	0.830	24 (10.8)
Beta blockers	13 (12.7)	12 (10)	0.531	12 (10.9)	13 (11.6)	1.000	25 (11.3)
ACEI or ARB	32 (31.4)	37 (30.8)	1.000	28 (25.5)	41 (36.6)	0.083	69 (31.1)
Metformin	13 (15.3)	18 (17.8)	0.595	19 (21.1)	12 (12.5)	0.620	31 (16.7)

Abbreviations: ACEI= Angiotensin converting enzyme inhibitor; AMI= Acute Myocardial Infarction; ARB= angiotensin receptor blocker; BMI= Body mass index; COPD= Chronic Obstructive Pulmonary Disease; RIC= Remote ischemic conditioning.

Table 3: Procedure details.

	RIC	Sham RIC	*P* Value	Exenatide	Matching Placebo	*P* Value	Total
Infarct-related artery, no.	102	119	0.478	110	111	0.934	221
Left anterior descending, no. (%)	35 (34.3)	45 (37.8)		38 (34.5)	42 (37.8)		80 (36.2)
Right coronary artery, no. (%)	47 (46.1)	54 (45.4)		53 (48.2)	48 (43.2)		101 (45.7)
Circumflex, no. (%)	10 (9.8)	11 (9.2)		11 (10)	10 (9)		21 (9.5)
Other, no. (%)	10 (9.8)	9 (7.6)		8 (7.3)	11 (9.9)		19 (8.6)
TIMI flow grade at admission							
TIMI=0, no. (%)	89 (87.3)	106 (89.1)		96 (88.1)	99 (88.4)		195 (88.2)
TIMI=1, no. (%)	13 (12.7)	13 (10.9)		13 (11.9)	13 (11.6)		26 (11.8)
Number of RIC cycles at reperfusion, total no.	102	118	0.931	109	111	0.960	220
1 cycles, no. (%)	5 (4.9)	6 (5.1)		6 (5.5)	5 (4.5)		11 (5)
2 cycles, no. (%)	41 (40.2)	51 (43.2)		43 (39.4)	49 (44.1)		92 (41.8)
3 cycles, no. (%)	38 (37.3)	35 (29.7)		40 (36.7)	33 (29.7)		73 (33.2)
4 cycles, no. (%)	18 (17.6)	26 (22)		20 (18.3)	24 (21.6)		44 (20)
Stenting of culprit lesion by PPCI, no. (%)	95 (96.9)	108 (96.4)	1.000	102 (98.1)	101 (95.3)	0.445	203 (96.7)
Aspiration thrombectomy, no. (%)	55 (56.7)	63 (57.8)	0.889	54 (52.9)	64 (61.5)	0.260	118 (57.3)

Table 3: Procedure details (continued)

	RIC	Sham RIC	*P* Value	Exenatide	Matching Placebo	*P* Value	Total
TIMI flow grade after procedure, total no.	102	118	0.131	109	111	0.788	220
TIMI=0, no. (%)	3 (2.9)	0 (0)		1 (0.9)	2 (1.8)		3 (1.4)
TIMI=1, no. (%)	0 (0)	1 (0.8)		1 (0.9)	0 (0)		1 (0.5)
TIMI=2, no. (%)	8 (7.8)	7 (5.9)		7 (6.4)	8 (7.2)		15 (6.8)
TIMI=3, no. (%)	91 (89.2)	110 (93.2)		100 (91.7)	101 (91)		201 (91.4)
Treatment at PPCI							
Heparin, no. (%)	77 (75.5)	97 (80.8)	0.414	85 (77.3)	89 (79.5)	0.746	174 (78.4)
Clopidogrel, no. (%)	67 (65.7)	76 (63.3)	0.779	69 (62.7)	74 (66.1)	0.674	143 (64.4)
Aspirin, no. (%)	101 (99)	117 (97.5)	0.627	108 (98.2)	110 (98.2)	1.000	218 (98.2)
Ticagrelor, no. (%)	19 (18.6)	26 (21.7)	0.618	25 (22.7)	20 (17.9)	0.406	45 (20.3)
Prasugrel, no. (%)	15 (14.7)	16 (13.3)	0.847	13 (11.8)	18 (16.1)	0.440	31 (14)
Nitrates, no. (%)	43 (42.2)	55 (45.8)	0.591	48 (43.6)	50 (44.6)	0.893	98 (44.1)

Table 3: Procedure details (continued)

	RIC	Sham RIC	*P* Value	Exenatide	Matching Placebo	*P* Value	Total
Number of patients, no.	102	120		110	112		222
CMR post PPCI, day no. (mean ± SD)	6±4.5	6.4±9.5	0.699	6.1±5.9	6.3±9	0.802	6.2±7.6
Primary Outcome							
Infarct size (%), (mean ± SD)[a]	24±11.8	23.7±10.9	0.827	25.1±11.5	22.5±10.9	0.092	23.8±11.3
Secondary Outcomes							
LVEDV (mL), (mean ± SD)	149.5±38.9	144.5±38	0.342	146.8±40.5	146.8±36.4	0.999	146.8±38.4
LVEDV/BSA, (mean ± SD)	78.7±17.9	75.4±17.9	0.167	76.2±18.5	77.6±17.4	0.550	76.9±17.9
LVESV (mL), (mean ± SD)	83.2±30.2	79.9±31.7	0.438	81.3±30.6	81.6±31.5	0.943	81.4±31
LVESV/BSA, (mean ± SD)	43.7±14.8	41.6±15.3	0.285	42.1±14.6	43.1±15.7	0.614	42.6±15.1
LVEF (%),(mean ± SD)	45.2±10.1	45.9±10.4	0.599	45.4±10.2	45.7±10.4	0.858	45.6±10.3
MSI (%),(mean ± SD) [b]	23.7±15.8	26.3±18.2	0.279	22.3±15.8	27.9±18.1	0.018	25.1±17.2
MVO (%),(mean ± SD) [c]	1±2.3	1.2±1.9	0.565	1.1±1.9	1±2.3	0.735	1.1±2.1
Transmularity Index, (mean ± SD) [d]	46.9±11.4	47.5±12.6	0.707	48.2±12.1	46.3±11.9	0.242	47.2±12
Extent of edema (gr), (mean ± SD)	37.2±16.3	37.9±16.9	0.751	37.9±17	37.3±16.3	0.780	37.6±16.6

Abbreviations: MSI=LGE/edema extent; MVO= Relative microvascular obstruction; PPCI= Primary percutaneous coronary intervention; RIC= Remote ischemic conditioning; SBP= Systolic blood pressure.

[a] Percentage of LGE volume to the total of left ventricular mass.

[b] Ratio of LGE to the extent of myocardial edema

[c] Percentage of dark areas of absent contrast surrounded by hyper-enhanced infarct tissue to the total Infarct size.

[d] Ratio of the mass of myocardium showing LGE to the mass of the myocardial segment containing it.

Table 4: Outcomes.

	RIC	Sham RIC	*P* Value	Exenatide	Matching Placebo	*P* Value	Total
Number of patients, no.	102	120		110	112		222
CMR post PPCI, day, mean ± SD	6±4.5	6.4±9.5	0.699	6.1±5.9	6.3±9	0.802	6.2±7.6
Primary Outcome							
Infarct size (%), mean ± SD [a]	24±11.8	23.7±10.9	0.827	25.1±11.5	22.5±10.9	0.092	23.8±11.3
Secondary Outcomes							
LVEDV (mL), mean ± SD	149.5±38.9	144.5±38	0.342	146.8±40.5	146.8±36.4	0.999	146.8±38.4
LVEDV/BSA, mean ± SD	78.7±17.9	75.4±17.9	0.167	76.2±18.5	77.6±17.4	0.550	76.9±17.9
LVESV (mL), mean ± SD	83.2±30.2	79.9±31.7	0.438	81.3±30.6	81.6±31.5	0.943	81.4±31
LVESV/BSA, mean ± SD	43.7±14.8	41.6±15.3	0.285	42.1±14.6	43.1±15.7	0.614	42.6±15.1
LVEF (%),mean ± SD	45.2±10.1	45.9±10.4	0.599	45.4±10.2	45.7±10.4	0.858	45.6±10.3
MSI (%), mean ± SD [b]	23.7±15.8	26.3±18.2	0.279	22.3±15.8	27.9±18.1	0.018	25.1±17.2
MVO (%), mean ± SD [c]	1±2.3	1.2±1.9	0.565	1.1±1.9	1±2.3	0.735	1.1±2.1
Transmularity Index, mean ± SD [d]	46.9±11.4	47.5±12.6	0.707	48.2±12.1	46.3±11.9	0.242	47.2±12
Extent of edema (gr), mean ± SD	37.2±16.3	37.9±16.9	0.751	37.9±17	37.3±16.3	0.780	37.6±16.6

Abbreviations: BSA= Body surface area; CMR=Cardiac magnetic resonance; LVEDV= Left Ventricular End Diastolic Volume; LVESV= Left Ventricular End Systolic Volume; LGE: late gadolinium enhancement; MSI=LGE/edema extent; MVO=Relative microvascular obstruction; PPCI= Primary percutaneous coronary intervention; RIC= Remote ischemic conditioning.

[a] Percentage of LGE volume to the total of left ventricular mass.

[b] Ratio of LGE to the extent of myocardial edema

[c] Percentage of dark areas of absent contrast surrounded by hyper-enhanced infarct tissue to the total Infarct size.

[d] Ratio of the mass of myocardium showing LGE to the mass of the myocardial segment containing it.

Table 5. Exploratory adverse events during hospitalization in the sample of patients included in the intention to treat analysis.

	RIC	Sham RIC	p	Exenatide	Matching Placebo	p	Total
Number of patients; n	102	120		110	112		222
Death; n (%)	1 (1)	2 (1.7)	1.000	1 (0.9)	2 (1.8)	1.000	3 (1.4)
Myocardial Infarction; n (%)	4 (3.9)	1 (0.8)	0.183	3 (2.7)	2 (1.8)	0.682	5 (2.3)
Stroke TIA; n (%)	0 (0)	3 (2.5)	0.252	1 (0.9)	2 (1.8)	1.000	3 (1.4)
Heart Failure; n (%)	3 (2.9)	4 (3.3)	1.000	4 (3.6)	3 (2.7)	0.720	7 (3.2)
New coronary intervention; n (%)	3 (2.9)	1 (0.8)	0.336	3 (2.7)	1 (0.9)	0.367	4 (1.8)
Ventricular arrhythmia during primary PCI; n (%)	12 (11.8)	11 (9.2)	0.659	11 (10)	12 (10.7)	1.000	23 (10.4)
Atrial fibrillation or flutter during hospitalization; n (%)	3 (2.9)	9 (7.5)	0.151	5 (4.5)	7 (6.3)	0.768	12 (5.4)
Local vascular complications; n (%)	3 (2.9)	2 (1.7)	0.663	3 (2.7)	2 (1.8)	0.682	5 (2.3)
Bradyarrhythmia requiring medical treatment; n (%)	8 (7.8)	6 (5)	0.418	5 (4.5)	9 (8)	0.409	14 (6.3)
Skin allergic reaction; n (%)	1 (1)	0 (0)	0.459	0 (0)	1 (0.9)	1.000	1 (0.5)

Abbreviations: MVO=Relative microvascular obstruction; TIA = transient ischemic attack.

Table 6. Exploratory adverse events during hospitalization in entire sample of randomized patients.

	RIC	Sham RIC	p	Exenatide	Matching placebo	p	Total
Number of patients; n	187	191		189	189		378
Death; n (%)	4 (2.1)	2 (1)	0.445	3 (1.6)	3 (1.6)	1.000	6 (1.6)
Myocardial Infarction; n (%)	7 (3.7)	2 (1)	0.102	3 (1.6)	6 (3.2)	0.503	9 (2.4)
Stroke TIA; n (%)	2 (1.1)	3 (1.6)	1.000	3 (1.6)	2 (1.1)	0.686	5 (1.3)
Heart Failure; (%)	4 (2.1)	9 (4.7)	0.259	6 (3.2)	7 (3.7)	0.787	13 (3.4)
New coronary intervention; n (%)	6 (3.2)	2 (1)	0.171	2 (1.1)	6 (3.2)	0.284	8 (2.1)
Ventricular arrhythmia (VT or VF) during PPCI; n (%)	14 (7.5)	12 (6.3)	0.688	13 (6.9)	13 (6.9)	1.000	26 (6.9)
Atrial fibrillation or flutter during hospitalization; n (%)	4 (2.1)	12 (6.3)	0.071	9 (4.8)	7 (3.7)	0.622	16 (4.2)
Local vascular complications in arterial access; n (%)	5 (2.7)	2 (1)	0.280	2 (1.1)	5 (2.6)	0.449	7 (1.9)
Bradyarrhythmia requiring medical treatment; n (%)	9 (4.8)	6 (3.1)	0.441	9 (4.8)	6 (3.2)	0.600	15 (4)
Skin allergic reaction; n (%)	1 (0.5)	0 (0)	0.495	1 (0.5)	0 (0)	1.000	1 (0.3)

Abbreviations: MVO= Relative microvascular obstruction; PPCI= Primary percutaneous intervention; TIA = Transient ischemic attack.

Figures 1-2

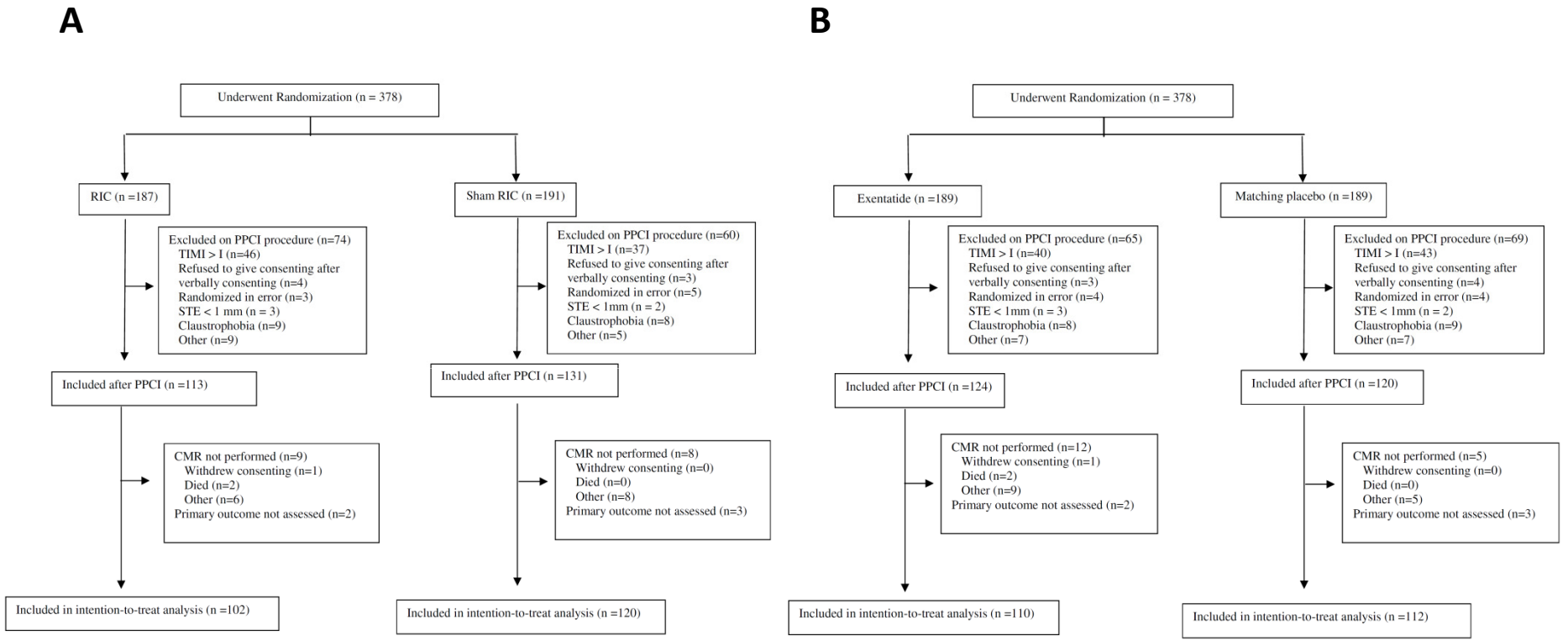

Figure 1

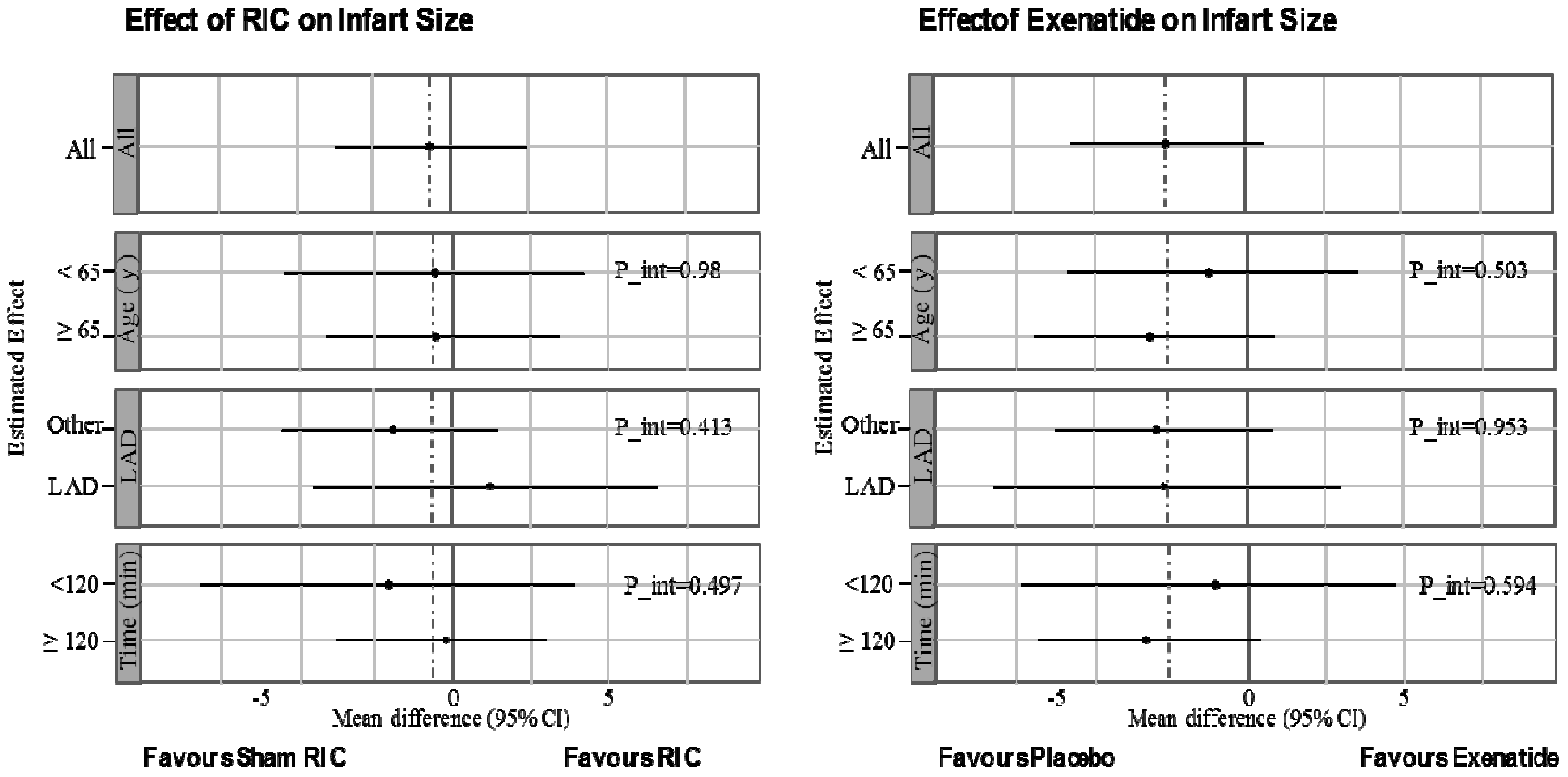
Effect of RIC on Infart Size
All
All
< 65
≥ 65
Age (y)
P_int=0.98
Other
LAD
LAD
P_int=0.413
<120
≥ 120
Time (min)
P_int=0.497
Estimated Effect
-5
0
5
Mean difference (95% CI)
Favours Sham RIC
Favours RIC
Effectof Exenatide on Infart Size
All
All
< 65
≥ 65
Age (y)
P_int=0.503
Other
LAD
LAD
P_int=0.953
<120
≥ 120
Time (min)
P_int=0.594
Estimated Effect
-5
0
5
Mean difference (95% CI)
Favours Placebo
Favours Exenatide

Figure 2

MANUSCRIPT # BRIC-D-20-00638

RESPONSES TO REVIEWER #1:

In the COMBAT trial, Garcia del Blanco et al. sought to determine whether the combination of Remote Ischemic Conditioning (RIC) and the GLP-1 analog exenatide might provide a greater infarct size reduction than placebo or each of these two interventions alone. They designed a two-by-two factorial protocol and performed a phase 2 randomized controlled, blinded, multicenter, clinical trial. The authors report no significant effect of either or combined interventions on infarct size as well as any of the secondary endpoints including recovery of LV function, MVO or arrhythmias.

This is a well-designed proof-of-concept clinical trial and one of a few to address the issue of combination of interventions aimed at reducing infarct size in STEMI patients. The results do not confirm previous studies showing a reduction of infarct either by RIC alone or by exenative alone and this has been mentioned and discussed in the manuscript. The presentation of the study protocol is a concern:

Answer: We thank the reviewer for his/her encouraging comments and useful suggestions. Please, find responses to specific comments below:

1. As written, it is unclear whether the RIC inflations were stopped or continued irrespective of coronary artery reopening by PPCI. As presented, it seems that the investigators stopped RIC, whatever the number of inflations performed, when the artery was to be reopened. This has to be clarified in the methods section. If this were the case, this is a pitty because RIC could have been completed during the first minutes of reflow (per-conditioning like) so that all patients receive the full RIC protocol.

Answer: We thank the reviewer for raising this critical issue, which was not clearly explained in the previous version of the manuscript. All patients completed the four cycles of cuff inflation/deflation. This information has been included in the methods section (page 6 (line 14) of the revised version of the manuscript). We provide a detailed description on the total number of cycles at the time of coronary blood flow restoration, as indicated in pages 12 (second paragraph) and 14 (lines 13-18). About half of the patients received 2 or fewer cycles before flow restoration, meaning that the remaining cycles were completed during the reperfusion phase in these patients. In any case, we analyzed independently the effect of the RIC protocol when at least 3 cycles were applied before flow restoration and no differences were observed between groups (see page 14, lines 13-18).

2. It is unclear when the exenatide infusion was started and stopped, in the exenatide alone group and in the (RIC + exenatide) group. How long before coronary artery reopening? How long after PPCI ?

Answer: Exenatide infusion was started before the procedure in all cases, i.e., before arterial access was obtained. This is now clarified in page 6 (third paragraph). Upon arrival at the cath lab and once the inclusion/exclusion criteria were checked patients were randomized and RIC was immediately applied by the cath lab personnel. At the same time, a nurse prepared and started the infusion. Meanwhile, a second nurse and the auxiliary nurse prepared the table to start the percutaneous procedure, to avoid any delay in reperfusion. Since the infusion volume of exenatide (or placebo) was 180 ml and the speed of perfusion was 72 ml/h during 15min and 26 ml/h thereafter, the total perfusion time lasted 6 hours 12 minutes. Although exenatide infusion started before coronary artery reperfusion in 100% of patients, the exact time at which exenatide infusion was started is not available.

3. P.14 : the authors state that :« Finally, the pattern of cardiac biomarkers release, as surrogate outcome of the IS, was not included, since it has been deemed inadequate for IS assessments [17] ». Two points :

3.a. According to Clinicaltrial.gov, release of cardiac enzymes has been measured in Vall d'Hebron. At least should the authors mentioned something about these data.

Answer: Following reviewer's suggestion we have included a subanalysis on the release of cardiac biomarkers at Vall d'Hebron Hospital. The description has been included in page 14, lines 21-27 of the revised version of the manuscript:

"In a subanalysis of 146 patients with valid data from the coordinator center, there were also no differences between the groups in the peak median values of CK-MB neither in the RIC hypothesis (median 261.85, Interquartile range [141.85 - 337.15] in the RIC group and 234.2 [174 - 353.6] in the sham group; p=0.43) nor in the exenatide hypothesis (266.1 [174 - 345.4] in the exenatide group and 229.95 [145.6 - 330.9] in the placebo group; p=0.33)".

3.b. The current wording is not correct. Either the authors concede that a surrogate, by definition, is an inadequate measure and then they rephrase this sentence (preferred option), or, they discuss why that cardiac enzyme release, widely use throughout the world, is not a good measurement of infarct size; then they would have to explain why CMR is better (I suggest not to go into this).

Answer: We have reworded this sentence according with the reviewer's suggestion (see page 14, lines 18-21):

"Finally, we decided not to include the pattern of cardiac biomarkers release as an endpoint because it is only a surrogate outcome of the actual IS and would require uniform measurement in a central laboratory to achieve good accuracy, which was not possible in this study.»

RESPONSES TO REVIEWER #2:

The COMBAT-MI trial is a two-by-two factorial, randomized controlled, blinded, multicenter, clinical trial investigating the effect of remote Ischemic Conditioning (RIC) and the GLP-1 analog exenatide against shame RIC/placebo on infarct size in beyond rapid revascularization in patients with ST-segment-elevation myocardial infarction. The primary outcome was infarct size measured by late gadolinium enhancement in cardiac magnetic resonance performed 3-7 days after PPCI. Secondary outcomes were myocardial salvage index, transmurality index, left ventricular ejection fraction and relative microvascular obstruction volume. A total of 378 patients were randomly allocated. After applying exclusion criteria, 222 patients were available for analysis. There were no significant interactions between the two randomization factors on the primary or secondary outcomes.

The study of a multitarget approach to cardioprotection beyond rapid revascularization is timely and appropriate because individual interventions, including RIC and exenatide, have demonstrated promising results in experimental and clinical proof-of-concept studies, while translation into a clinical benefit has - at least for RIC - shown variable results.

The study appears well conducted but some concerns remain.

1) The premises for the sample size calculation is not quite clear in the manuscript nor in the description on clinicaltrials.gov. It is stated that sample size has been calculated to be 274 patients with TIMI 0-1 available for analysis of the primary end-point. What were the assumptions to reach this number? The assumption to calculate the number needs to include anticipated infarct size and SD. I mention this because previous studies investigating interventions modulating infarct size often use salvage index as primary endpoint and based on these studies relying on a simple 1:1 randomization the needed patient numbers vary between 69 and 90 in each study group (e.g. J Am Coll Cardiol. 1995; 26: 1657-1664 (**Schroder**), Lancet. 2002; 359: 920-925 (**Kastrati**), Circulation. 2006;114:40-47 (**Kaltoft**), Lancet 2010; 375:727-734 (**Botker**)). The use of salvage index takes advantage of relating infarct size to area at risk such that interindividual variability is eliminated, which is not the case for infarct size. A rough power calculation using the authors own data (20% reduction from infarct size 24% of LV and SD 12%) indicate study group sizes of at least 90 and perhaps even higher if you use smaller infarct size (i.e. 119 patients in each group with infarct size of 23 and SD 11). Even though it is surprising that the interindividual variability by the use of infarct size does not seem to be of so much importance as I expected, the authors seem to be relatively safe with their numbers probably because infarct sizes were relatively high and interindividual variability low as assessed from the SDs. However, also the 2 x 2 factorial design might modify requirements to the statistical power (I would expect a need for a higher number of patients). I fully accept that salvage index is not a useful endpoint using CMR and I also accept that the authors have already mentioned the limitation of not reaching the target number but specifications about the original power calculation should be addressed.

Answer: We thank the reviewer for his/her positive comments and insight. We agree that the method used to calculate sample size was not adequately explained in the previous version of our manuscript. The essential assumption, based on previous trials, was a mean of IS around

24% with a standard deviation of 14. Assuming a significance level alfa=0.05 and a statistical power (1-beta) = 0.8, a total sample size 274 patients would be necessary to detect a minimum effect on IS reduction of 20% with either exenatide or RIC or both treatments.

To reflect properly this concept we have adapted the text in the revised version of the manuscript (page 8, lines 21-25):

"*To calculate sample size, we assume a conservatively IS of 24% with a standard deviation of 14 based on previous studies. Sample size was computed to detect a reduction of IS of at least 20% with either RIC or exenatide or with the combined therapy, with a statistical power 1-β= 0.8 and a significance level α = 0.05. This resulted in a calculated total sample size of 274 patients."*

Concerning the selection of the primary outcome: The selection of infarct size as the primary outcome rather than MSI was based on a larger expected error in estimating the latter. And this is so because the estimation of the area at risk requires the use of STIR sequences in cardiac magnetic resonance imaging, which are more subject to measurement artifacts when comparing late enhancement sequences. These latter sequences provide higher quality images and therefore necrosis area is much easier to delineate, making infarct size estimation much more robust. In fact, standard deviations are usually lower in infarct size. As a real example, our group has published our inter-observer variability in the estimation of both area of necrosis and area at risk, achieving a higher CCI in the former: 0.89 (0.82-0.93) and 0.81 (0,70-0.89) respectively. Rev Esp Cardiol. 2012;65(11):1010–1017.

2) As a continuation of the power concern, it would help the reader to include a proper 2 x 2 factorial table specifying the number of patients in each study group.

Answer: The exact treatment distribution in a 2X2 factorial table was:

Placebo exenatide plus RIC: n=54

Placebo exenatide plus sham RIC: n=58

Exenatide plus RIC: n=48

Exenatide plus sham RIC: n= 62

These data have been included in the new table 1 of the revised version of the manuscript.

3) As a further continuation I was surprised that the SDs of the MSI was higher than the SDs of final infarct size because the use of MSI should eliminate interindividual variability while the use of infarct size does not.

Answer: We have addressed this important point in the paragraph "1".

4) Given that the majority of patients had not had previous MI and Killip class was I in the majority of patients, overall mean infarct size seemed somewhat high and higher than in many

previous studies using CMR for infarct size quantification in STEMI patients undergoing PPCI (e.g. Eur Heart J 2012;33:1491-9 but also others). While this is an advantage for the power of the study, it would be appropriate to provide some explanation for these infarct sizes, which are also higher than seen when SPECT is used for quantification (e.g. Circulation. 2006;114:40-47, Lancet 2010; 375:727-734)? What was the delay from symptom onset/first medical contact to balloon time?

Answer: As the Reviewer pointed out, the percentage of patients in Killip class I was similar among studies, as was the percentage of anterior infarctions. However, ischemic time was not larger in our study (median time between onset of symptoms and arrival to the cath lab 137 [IQR 101-180] min) than in those studies mentioned by the Reviewer, which suggests that this is not the explanation for the observed discrepancy in infarct size. Instead, there are technical differences in infact size estimation among studies that may have contributed to the differences in infarct size. First, infarct size measurements by SPECT and CMR are not comparable because the spatial resolution of the latter technique is higher (Wagner et al, Lancet 2003:361, 374-379). With regard to CMR studies, the timing of the exam and the details of image acquisition may have a significant influence on infarct size estimation. As time passes, infarct volume decreases due to reduction of edema and to the healing process (Ibrahim et al. Radiology 2010; 254:88). Of note, in the study mentioned by the Reviewer (Eur Heart J 2012;33:1491-9), infarct size ("final infarct size") was measured 90 ± 21 days after the index event, whereas in our study it was measured in the first days. Other technical factor that could underlie these differences is the time between CMR contrast administration and image acquisition (Rodriguez-Palomares, J Cardiovasc Magn Reson, 2015:17:43), although the contribution of this factor seems unlikely in our study because it was set to 25 min to minimize its influence.