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ORIGINAL ARTICLE

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Remote ischaemic preconditioning in isolated aortic valve and coronary artery bypass surgery: a randomized trial†

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TRANSLATIONAL RESEARCH

Key question

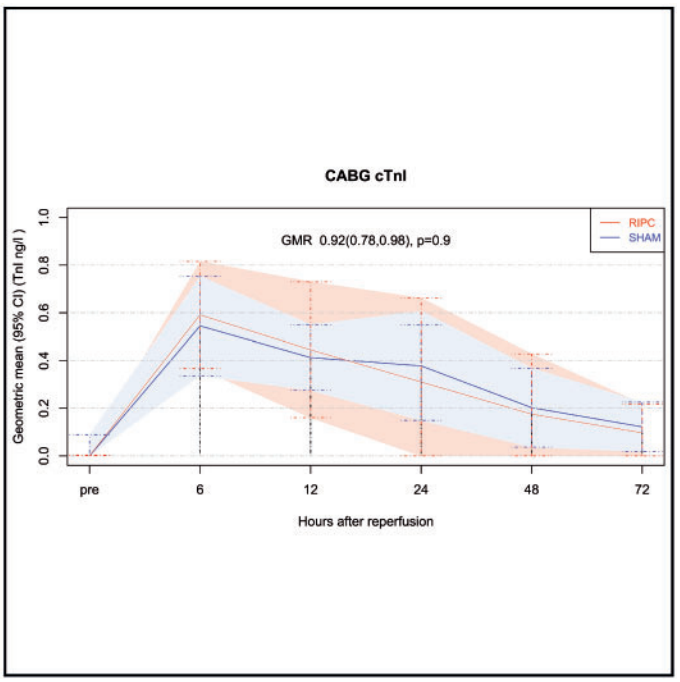
Does remote ischaemic preconditioning (RIPC) confer cardioprotection in patients undergoing CABG or aortic valve replacement (AVR) surgery?

Key findings

RIPC does not ameliorate cardiac injury, metabolic stress, and inflammatory response during CABG or AVR surgery.

Take-home message

This trial supports the view that RIPC does not confer additional cardioprotection in patients undergoing isolated CABG or AVR surgery.



Abstract

OBJECTIVES: This trial was designed and patients were recruited at a time when the benefits of remote ischaemic preconditioning during open-heart surgery were still controversial. We focused on a homogeneous patient population undergoing either isolated aortic valve replacement or coronary artery bypass grafting (CABG) surgery by investigating cardiac injury, metabolic stress and inflammatory response.

METHODS: A 2-centre randomized controlled trial recruited a total of 124 patients between February 2013 and April 2015. Of them, 64 patients underwent CABG and 60 patients underwent aortic valve replacement. Patients were randomized to either sham or preconditioning. Remote ischaemic preconditioning was applied following anaesthesia and before sternotomy. Myocardial injury and inflammatory response were assessed by serially measuring cardiac troponin I, and interleukin-6, 8, 10 and the tumour necrosis factor (TNF-α). Biopsies from the left and the right ventricles were harvested after ischaemic reperfusion injury for nucleotides analysis.

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RESULTS: Application of remote ischaemic preconditioning did not alter the degree of troponin I release, levels of inflammatory markers and cardiac energetics in both the CABG and the aortic valve replacement groups.

CONCLUSIONS: Preconditioning did not confer any additional cardioprotection in terms of reducing the levels of troponin I and inflammatory markers and preserving left and right ventricle energy metabolites in patients undergoing isolated CABG or aortic valve surgery.

Clinical trial registration number: International Standard Randomized Controlled Trial Number (ISRCTN) registry ID 33084113 (doi: 10.1186/ISRCTN33084113) and UK controlled randomized trial number (UKCRN) registry ID 13672.

Keywords: Remote ischaemic preconditioning • Coronary artery bypass grafting • Aortic valve replacement • Cardiac injury

INTRODUCTION

Two recent large randomized trials have shown neither troponin reduction nor clinical benefit in patients undergoing cardiac surgery after upper limb remote ischaemic preconditioning (RIPC) [1, 2]. Different confounders, heterogeneous population of patients with different pathologies and a variety of comorbidities may have significantly biased the efficacy of the intervention [3].

The mechanisms underlying RIPC protection in experimental models and clinical setting are still poorly understood [4]. Work in experimental models has monitored changes in cardiac metabolites in the ventricular biopsies to help understand how this intervention is working [5]. Additionally, knowledge of the potential RIPC-induced changes in systemic stress (e.g. inflammatory response) would also help in elucidating the effects of RIPC during cardiac surgery [4].

Thus, the aim of this trial was to investigate the effect of the upper limb RIPC in patients undergoing isolated coronary artery bypass grafting (CABG) and aortic valve replacement (AVR) on cardiac injury, metabolic stress and inflammatory response.

PATIENTS AND METHODS

Trial design

A 2-centre randomized controlled trial investigating the effects of RIPC in patients undergoing (i) isolated CABG and (ii) isolated AVR with cardiopulmonary bypass (CPB) and cardioplegic arrest. The research objectives were addressed by randomizing participants within each of the 4 patient strata to RIPC or SHAM control. The randomization was carried out by a research nurse, who carried out both interventions in the theatre. Participants, clinicians and trial personnel were blinded to which group a participant was assigned. The study was conducted at the Hammersmith Campus of Imperial College Healthcare NHS Trust and the University Hospitals of Bristol NHS Foundation Trust. The study was approved by the London-Harrow Research Ethics Committee (reference number REC number 12/LO/1361) and was registered to the International Standard Randomized Controlled Trial Number (ISRCTN) registry with the ID 33084113 (doi: 10.1186/ISRCTN33084113) and to the UK controlled randomized trial number (UKCRN) registry ID 13672. The study was sponsored by the Imperial College of London and supported by the British Heart Foundation (BHF) and the Biomedical Research Unit (BRU) [6]. The recruitment was carried out from February 2013 to April 2015 (Supplementary Material, the CONSORT checklist).

Participants and remote ischaemic preconditioning protocol

Inclusion and exclusion criteria, and trial conduct were published previously [6] (Supplementary Material). RIPC was induced as described by others [1, 7]. Briefly, RIPC comprised four 5-min cycles of upper limb ischaemia, induced by a blood pressure cuff inflated to 200 mmHg, with an intervening 5 min of reperfusion by deflating the cuff.

Outcomes

The study's primary end point was troponin I [cardiac troponin I (cTnI)] measured at baseline (before the operation) and 6 h, 12 h, 24 h, 48 h and 72 h after the aortic cross-clamp release. Secondary outcomes were myocardial metabolites measured in snap-frozen biopsies obtained with a Trucut needle from the left ventricle and the right ventricle 20 min after the index ischaemia (aortic cross-clamp); the blood inflammatory markers: interleukin (IL)-6, 8, 12 and tumour necrosis factor (TNF)-α; blood pH; systemic metabolic stress assessed by lactate and serum creatinine level. Inflammatory markers, lactate and pH were measured at the same time points as for cTnI; serum creatinine was measured at the baseline and from postoperative days 1–7. Relevant clinical outcomes and serious adverse events were also recorded.

Sample size and statistical analysis

Sample size was estimated from our previous work [6] (Supplementary Material). Analyses were performed on an intention-to-treat basis. The Shapiro–Wilk's test was used to check the normality of data in groups before further analysis. Continuous data are summarized as mean ± standard deviation or median (interquartile range) if distributions are skewed. Categorical data are summarized as number and percentage. Repeated measures (troponin, inflammatory markers, pH, lactate and creatinine) were compared using the linear mixed model (fixed: intervention, time, interaction intervention and time; random: time crossed with subject within treatment). Outcomes analysed on a logarithmic scale were transformed back to the original scale after analysis, and the results were presented as geometric mean ratios (GMRs) as previously described [8]. Ventricle biopsies were compared using the unpaired, 2-tailed, *t*-test or the Wilcoxon rank sum test; *P*-value <0.05 was considered significant. Energy charge was calculated as follows: energy charge = ATP + (0.5 × ADP)/ATP + ADP + AMP [5].

Table 1: Baseline characteristics

	CABG			AVR			All ($n = 124$)
	RIPC ($n = 32$)	SHAM ($n = 32$)	Overall ($n = 64$)	RIPC ($n = 31$)	SHAM ($n = 29$)	Overall ($n = 60$)	
Age (years)	63.4 ± 8.9	62.9 ± 16.3	64.1 ± 10.3	71.4 ± 16.8	66.2 ± 12.6	68.9 ± 15	66.4 ± 13
Male gender	28 (87.5)	27 (84.3)	55 (85.9)	19 (61.3)	20 (69)	39 (65)	94 (75.8)
Body mass index (kg/m^2)	28.2 ± 4.5	29.2 ± 5.6	28.7 ± 5.1	29.2 ± 6.8	28.7 ± 5.5	29 ± 6.2	28.8 ± 5.6
NYHA							
I	10 (31.2)	12 (37.5)	22 (34.4)	19 (61.3)	20 (69)	39 (65)	61 (49.2)
II	17 (53.1)	18 (56.2)	35 (54.7)	4 (12.9)	2 (6.9)	6 (10)	41 (33)
III	4 (12.5)	2 (6.2)	6 (9.3)	17 (54.8)	19 (65.5)	36 (60)	42 (33.9)
IV	1 (3.1)	0	1 (1.6)	10 (32.2)	8 (27.6)	18 (30)	19 (15.3)
CCS							
I	9 (28.1)	8 (25)	17 (26.6)	4 (12.9)	6 (20.7)	10 (16.6)	27 (21.7)
II	17 (53.1)	19 (59.4)	36 (56.2)	10 (32.2)	7 (24.1)	17 (28.3)	53 (42.7)
III	5 (15.6)	3 (9.3)	8 (12.5)	2 (6.4)	0	2 (3.3)	10 (8)
IV	0	0	0	0	1 (3.4)	1 (1.6)	1 (0.8)
Previous MI	3 (9.3)	13 (40.6)	16 (25)	2 (6.4)	0	2 (3.3)	18 (14.5)
AF	0	0	0	2 (6.4)	1 (3.4)	3 (5)	3 (2.4)
Permanent pacemaker	1 (3.1)	1 (3.1)	2 (3.1)	1 (3.2)	1 (3.4)	2 (3.3)	4 (3.2)
LV good >50%	26 (81.2)	23 (71.8)	49 (79.6)	26 (83.9)	26 (89.6)	52 (86.6)	101 (81.4)
LV moderate <50% and >30%	6 (18.7)	9 (28.1)	15 (23.4)	4 (12.9)	3 (10.3)	7 (11.6)	22 (17.7)
LV less <30%	0	0	0	1 (3.2)	0	0	1 (0.8)
Smoking	15 (46.9)	17 (53.1)	32 (50)	15 (48.4)	10 (34.5)	25 (41.6)	57 (46)
Ex-smoking	5 (15.6)	2 (6.2)	8 (12.5)	1 (3.2)	6 (20.7)	7 (11.6)	15 (12)
Family history CAD	24 (75)	23 (71.8)	47 (73.4)	12 (38.7)	13 (44.8)	25 (41.6)	72 (58)
Hypercholesterolaemia	29 (90.6)	31 (96.9)	60 (93.7)	16 (51.6)	17 (58.6)	33 (55)	93 (75)
Hypertension	26 (81.2)	30 (93.7)	56 (87.5)	23 (74.2)	20 (68.9)	43 (71.6)	99 (79.8)
Hypothyroidism	0	1 (3.1)	1 (1.6)	3 (9.7)	3 (10.3)	6 (10)	7 (5.6)
COPD	5 (15.6)	7 (21.9)	12 (18.7)	3 (9.7)	3 (10.3)	6 (10)	18 (14.5)
CVA/TIA (%)	4 (12.5)	1 (3.1)	5 (7.8)	3 (9.7)	3 (10.3)	6 (10)	11 (8.9)
Neurological dysfunction	0	0	0	0	1 (3.4)	1 (1.6)	1 (0.8)
IDDM	1 (3.1)	3 (9.3)	4 (6.25)	0	1 (3.4)	1 (1.6)	5 (4)
NIDDM	5 (15.6)	13 (40.6)	18 (28.1)	4 (12.9)	4 (13.8)	8 (13.3)	26 (21)
Extracardiac arteriopathy	1 (3.1)	0	1 (1.6)	1 (3.2)	2 (6.9)	3 (5)	4 (3.2)
Creatinine (mg/dl)	82 ± 14	95.1 ± 20.7	88.6 ± 18.7	87.3 ± 22	78.6 ± 16.2	83.1 ± 19.7	85.9 ± 19.3
Number of CABG	2.7 ± 0.5	2.7 ± 0.5	2.7 ± 0.5	0.1 ± 0.7	0 ± 0	0.08 ± 0.5	1.4 ± 1.4
Elective	28 (87.5)	30 (9.4)	58 (90.6)	29 (936)	27 (93.1)	56 (93.3)	114 (92)
Urgent	4 (12.5)	2 (6.2)	6 (9.4)	2 (6.4)	2 (6.9)	4 (6.6)	10 (8)

Values are presented as median (first and third quartiles), mean ± SD or *n* (%).

AF: atrial fibrillation; AVR: aortic valve replacement; CABG: coronary artery bypass grafting; CAD: coronary artery disease; CCS: Canadian Cardiovascular Society; COPD: chronic obstructive pulmonary disease; CVA: cerebral vascular accident; IDDM: insulin-dependent diabetes mellitus; LV: left ventricular function; MI: myocardial infarction; NIDDM: non-insulin-dependent diabetes mellitus; NYHA: New York Heart Association; RIPC: remote ischaemic preconditioning; SD: standard deviation; TIA: transient ischaemic attack.

The trial was not powered to detect differences in clinical outcomes, and their frequencies are descriptively tabulated. All analyses and data visualization were performed in the R Core Team. R: A Language and Environment for Statistical Computing; http://www.R-project.org (packages used: 'aov', 'graphics', 'lmer' and 'stat'). Vienna, Austria: R Foundation for Statistical Computing; 2014.

Anaesthesia and surgical management

Anaesthetic, CPB, cardioplegia and surgical techniques and any other aspects of pre- and postoperative management were in accordance with existing protocols in use at both centres (Supplementary Material) as previously reported [6, 8].

RESULTS

Recruitment

Between February 2013 and April 2015, 316 patients were screened at the Hammersmith Hospital and Bristol Royal Infirmary Hospital for inclusion in the trial. Sixty-four patients were ineligible (see CONSORT diagram, Supplementary Material, Fig. S1). Of the 252 eligible patients, 124 patients agreed to participate in the study; 64 and 60 patients formed the CABG and AVR population, respectively, which was randomized to RIPC/SHAM. The primary analysis includes all randomized participants. Participants were followed up for 3 months after randomization. Safety data at 3 months were available for all the participants.

Baseline data

Overall baseline data are reported in Table 1. In the CABG group, patients allocated to SHAM when compared with those allocated to RIPC included more individuals with previous myocardial infarction (MI; 40.6% vs 9.3%), type 2 diabetes mellitus (NIDDM) (40.6% vs 15.6%) and higher creatinine (95.1 ± 20.7 and 82 ± 14). In the AVR group, patients allocated to RIPC compared with those allocated to SHAM had slightly higher creatinine (87.3 ± 22 and 78.6 ± 16.2). Four patients underwent unplanned concomitant CABG. The surgeons performing the procedures on reviewing the coronary angiogram after randomization felt that the

Table 2: Intraoperative details

	CABG			AVR		
	RIPC (*n* = 32)	SHAM (*n* = 32)	Overall (*n* = 64)	RIPC (*n* = 31)	SHAM (*n* = 29)	Overall (*n* = 60)
Operation duration (h)	3.6 (3–4)	3.7 (3.3–4.1)	3.7 (3.2–4.1)	3.1 (2.9–3.5)	3.1 (2.9–3.4)	3.1 (2.9–3.4)
CCT (min)	40.5 (32.5–49.5)	42 (33–49)	42 (32.5–49.5)	66 (55–79)	63 (52–85)	64 (52–81)
CPB (min)	77.5 (71.5–85.5)	85 (68.5–95)	79.5 (70.5–94)	93 (75–112)	91 (80–110)	93 (77.5–111)
DC shock after CC release	3 (9.3)	1 (3.1)	4 (6.2)	6 (18.7)	7 (24.1)	13 (21.6)
SR after CC release	28 (87.5)	28 (87.5)	56 (87.5)	20 (64.5)	20 (68.9)	40 (66.6)
IABP	1 (3.1)	0	1 (1.5)	1 (3.2)	1 (3.4)	2 (3.3)
Use of tranex	23 (71.8)	23 (71.8)	46 (71.8)	27 (87)	25 (86.2)	52 (86.6)
Intraoperative RBC	0.06 ± 0.3	0.6 ± 1	0.3 ± 0.8	0.2 ± 0.6	0.03 ± 0.1	0.1 ± 0.3
Noradrenalin	14 (43.7)	11 (34.3)	25 (39)	6 (19.3)	5 (17.2)	11 (18.3)
Dobutamine	0	0	0	0	0	0
Enoximone	1 (3.1)	0	1 (1.5)	3 (9.6)	2 (6.9)	5 (8.3)
Need for a pacemaker	1 (3.1)	1 (3.1)	2 (3.1)	5 (16.1)	6 (20.6)	11 (18.3)
Number of CABG	4.4 ± 0.8	4.1 ± 0.9	4.2 ± 0.9			

Values are presented as median (first and third quartiles), mean ± SD or *n* (%).

AF: atrial fibrillation; AV: atrioventricular; AVR: aortic valve replacement; CABG: coronary artery bypass grafting; CC: cross-clamp; CCT: cross-clamp time; CPB: cardio pulmonary bypass; Cryo: cryoglobulin; FFP: fresh frozen plasma; IABP: intra-aortic balloon pumping; PLT: platelets; RBC: red blood cells; RIPC: remote ischaemic preconditioning; SD: standard deviation; VF: ventricle fibrillation; VT: ventricle tachycardia.

degree of coronary stenosis was significant, hence, additional CABG was needed. Those patients were included as per the intention-to-treat analysis (3 patients in the RIPC group and 1 patient in the SHAM group). Preoperative medications are reported in the Supplementary Material, Table S1.

Operative details

Operative details are illustrated in Table 2. In both CABG and AVR, there was no difference in the RIPC group or the SHAM group in the cross-clamp time, the CPB time and the overall duration of the surgical procedure. There was no in-hospital mortality. Postoperative complications are described in Tables 3 and 4. No serious adverse events were recorded.

Cardiac troponin I release

The coronary artery bypass grafting group. Troponin I concentrations are illustrated in Fig. 1A and summarized in the Supplementary Material, Table S2. Preoperative concentrations were similar in the 2 groups [30 of 32 (93.7%) below the detectable limit, median concentration of 0.25 ng/l among participants with detectable concentrations in the RIPC group vs 30 of 32 (93.7%) and 0.007 ng/l in the SHAM group]. cTnI concentrations increased following surgery peaking at 6 h and were, on average, 8% lower in the RIPC group [GMR 0.92 (0.78–0.98), $P = 0.24$].

Creatinine, pH and lactate concentration are also illustrated in Fig. 1B–D. Postoperative creatinine, lactate concentrations and blood pH did not significantly differ between the groups; the postoperative creatinine concentration was lower in the RIPC group [GMR 0.85; 95% confidence interval (CI) 0.83–0.86; $P = 0.74$]; pH was slightly lower in the SHAM group (mean difference, 1; 95% CI 0.99–1; $P = 0.26$) and lactate concentrations were, on average, 7% lower in the RIPC group (GMR 0.93; 95% CI 0.87–0.99; $P = 0.9$). No serious adverse events were recorded.

The aortic valve replacement group. Troponin I concentrations are illustrated in Fig. 2A and summarized in the Supplementary Material, Table S3. The preoperative concentrations were similar in the 2 groups: [31 of 31 (100%) below the detectable limit in the RIPC group vs 28 of 29 (96.5%) in the SHAM group]. cTnI concentrations increased following surgery peaking at 6 h and were, on average, 10% lower in the SHAM group [GMR 1.1 (0.65–1.44), $P = 0.65$].

Table 3: CABG: postoperative details

	Randomized to RIPC (*n* = 32)	Randomized to SHAM (*n* = 32)	Overall (*n* = 64)
Total ventilation time (h)	8 (6–12)	7.5 (5–10)	7.7 (5–12)
Time in the ICU (h)	15 (12–18)	9 (3.6–14.4)	12 (8.1–17.1)
Time in the ward (h)	3 (2–5)	4 (3–5)	3 (3–5)
Length of hospital stay (days)	6.5 (6–8)	6.5 (6–8)	6.5 (6–8)
In-hospital mortality	0	0	0
Myocardial infarction	1 (3.1)	0	1 (1.5)
ST/AF	5 (15.6)	5 (15.6)	10 (15.6)
VF/VT	0	0	0
Pacing permanent (%)	0	0	0
Reopening for bleeding (%)	0	1 (3.1)	1 (1.5)
Inotropes used	17 (53.1)	15 (23.4)	(50)
IABP	0	0	0
Vasodilators used	8 (25)	6 (18.7)	(21.8)
Low cardiac output	0	0	0
Reintubation	0	0	0
Tracheostomy	0	0	0
C-PAP mask	9 (28.1)	6 (18.7)	15 (18.7)
Pneumothorax/effusion	2 (6.2)	3 (9.3)	5 (7.8)
Respiratory infection	1 (3.1)	1 (3.1)	2 (3.1)
Haemofiltration/dialysis (%)	0	0	0
Permanent stroke/TIA	0	0	0

Values are presented as median (first and third quartiles) or *n* (%). There were no missing data.

AF: atrial fibrillation; CABG: coronary artery bypass grafting; IABP: intra-aortic balloon pump; ICU: intensive care unit; RIPC: remote ischaemic preconditioning; TIA: transient ischaemic attack; VF: ventricle fibrillation; VT: ventricle tachycardia.

Postoperative creatinine, blood pH and lactate concentrations were similar in both groups (Fig. 2B–D), as was the postoperative creatinine concentration (GMR 1.07; 95% CI 0.97–1.17; $P = 0.56$); pH was, on average, 10% lower in the RIPC group (the mean difference 0.9; 95% CI 0.89–1; $P = 0.85$), whereas lactate was slightly lower in the SHAM group (GMR 1.01; 95% CI 0.89–1.27; $P = 0.63$).

Table 4: AVR: postoperative details

	Randomized to RIPC ($n = 31$)	Randomized to SHAM ($n = 29$)	Overall ($n = 60$)
Total ventilation time (h)	8 (4–12)	7.2 (5–13)	7.5 (4–13)
Time in the ICU (h)	13.2 (9–24)	12 (4.5–20)	12 (6.6–21)
Time in the ward (h)	3.5 (3–6)	3 (2–5)	3 (2.5–6)
Length of hospital stay (days)	7 (6–9)	6 (5–8)	7 (6–8.5)
In-hospital mortality	0	0	0
Myocardial infarction	0	0	0
Tachycardia/AF	18 (58)	12 (38.7)	30 (50)
VF/VT	0	0	0
Pacing permanent	2 (6.4)	0	2 (3.3)
Reopening for bleeding	1 (3.2)	0	1 (1.6)
Inotropes used	15 (48.3)	17 (58.6)	(53.3)
IABP	0	0	0
Vasodilators used	13 (41.9)	10 (34.4)	23 (38.3)
Low cardiac output	1 (3.2)	0	1 (1.6)
Reintubation	0	1 (3.4)	1 (1.6)
Tracheostomy	0	1 (3.4)	1 (1.6)
C-PAP mask	5 (16.1)	1 (3.4)	6 (10)
Pneumothorax/effusion	1 (3.2)	1 (3.4)	2 (3.3)
Respiratory infection	4 (12.9)	4 (13.7)	8 (13.3)
New haemofiltration/dialysis	0	0	0
Permanent stroke/TIA	0	0	0

Values are presented as median (first and third quartiles) or *n* (%). There were no missing data.

AF: atrial fibrillation; AVR: aortic valve replacement; IABP: intra-aortic balloon pump; ICU: intensive care unit; RIPC: remote ischaemic preconditioning; TIA: transient ischaemic attack; VF: ventricle fibrillation; VT: ventricle tachycardia.

Inflammatory markers

The expression of relevant cytokines was assessed using the MILLIPLEX® MAP Human High Sensitivity T Cell Magnetic Bead Panel (Supplementary Material).

The coronary artery bypass grafting group. IL-6, 8, 10 and TNF-α baseline and postoperative values for both the groups are depicted in Fig. 3. There were no statistically significant differences for each inflammatory marker considered ($P = 0.62$, 0.72, 0.73 and 0.81, IL-6, 8, 10 and TNF-α RIPC versus SHAM, respectively).

The aortic valve replacement group. IL-6, 8, 10 and TNF-α baseline, and postoperative values for both the groups are depicted in Fig. 4. There were no differences for each inflammatory marker considered ($P = 0.84$, 0.43, 0.5 and 0.28, IL-6, 8, 10 and TNF-α RIPC versus SHAM, respectively).

Cardiac metabolites

The metabolites measured were adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP). They were measured using the high-performance liquid chromatography as previously described [5] (Supplementary Material). The specimens were all of high quality with wet weight of 2.6 (1.8) mg and 2.9 (2) mg for CABG and AVR, respectively.

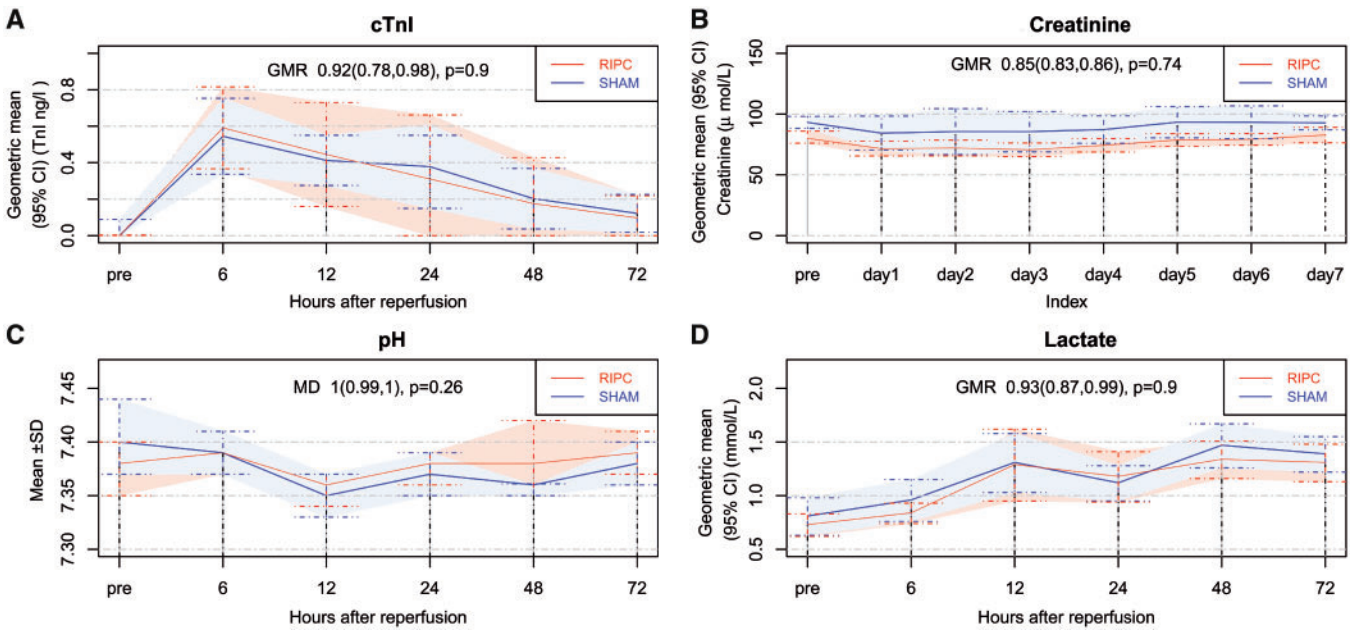

Figure 1: Coronary artery bypass grafting: concentration over time. Geometric mean and 95% CI at each study time point by group, and GMR and 95% CI for the effect of RIPC versus SHAM on (**A**) cTnI, (**B**) creatinine, (**C**) pH and (**D**) lactate. Mean and SD at each study time point by group, and MD and 95% CI for the effect of RIPC versus SHAM on pH level. CI: confidence interval; cTnI: cardiac troponin I; GMR: geometric mean ratio; MD: mean difference; pre: preoperative; RIPC: remote ischaemic preconditioning; SD: standard deviation.

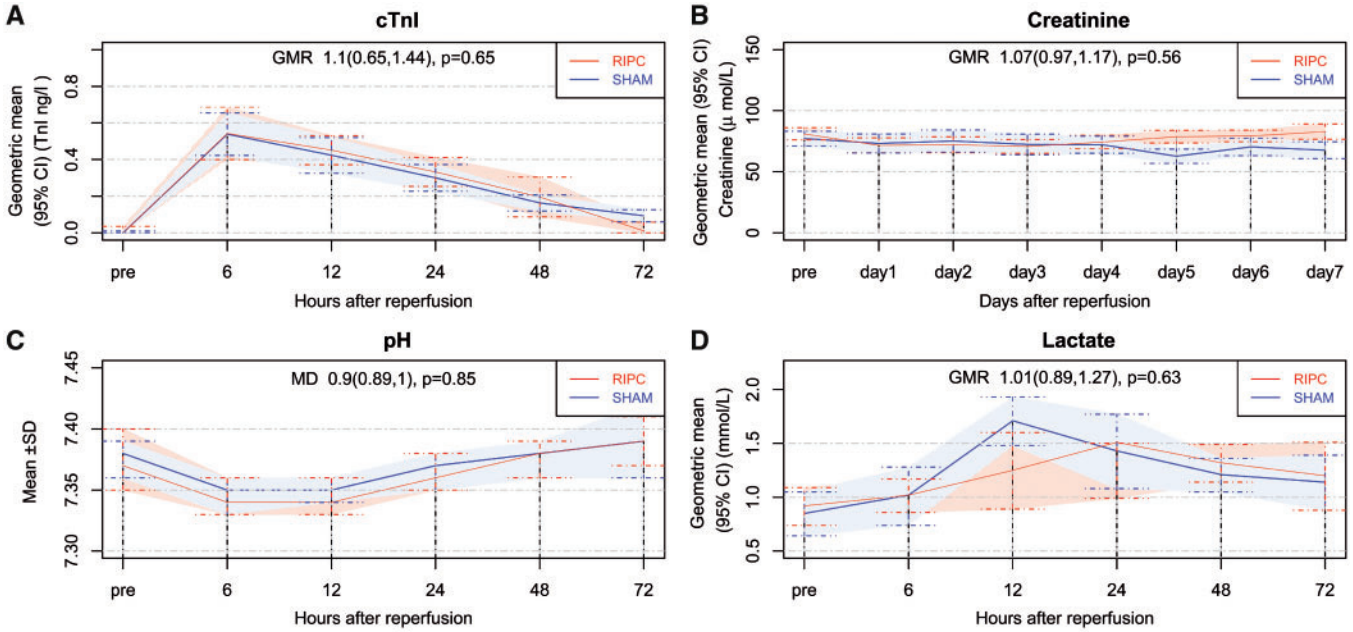

Figure 2: Aortic valve replacement: concentration over time. Geometric mean and 95% CI at each study time point by group, and GMR and 95% CI for the effect of RIPC versus SHAM on (**A**) cTnI, (**B**) creatinine, (**C**) pH and (**D**) lactate. Mean and SD at each study time point by group and MD and 95% CI for the effect of RIPC versus SHAM on pH level. CI: confidence interval; cTnI: cardiac troponin I; GMR: geometric mean ratio; MD: mean difference; pre: preoperative; RIPC: remote ischaemic preconditioning; SD: standard deviation.

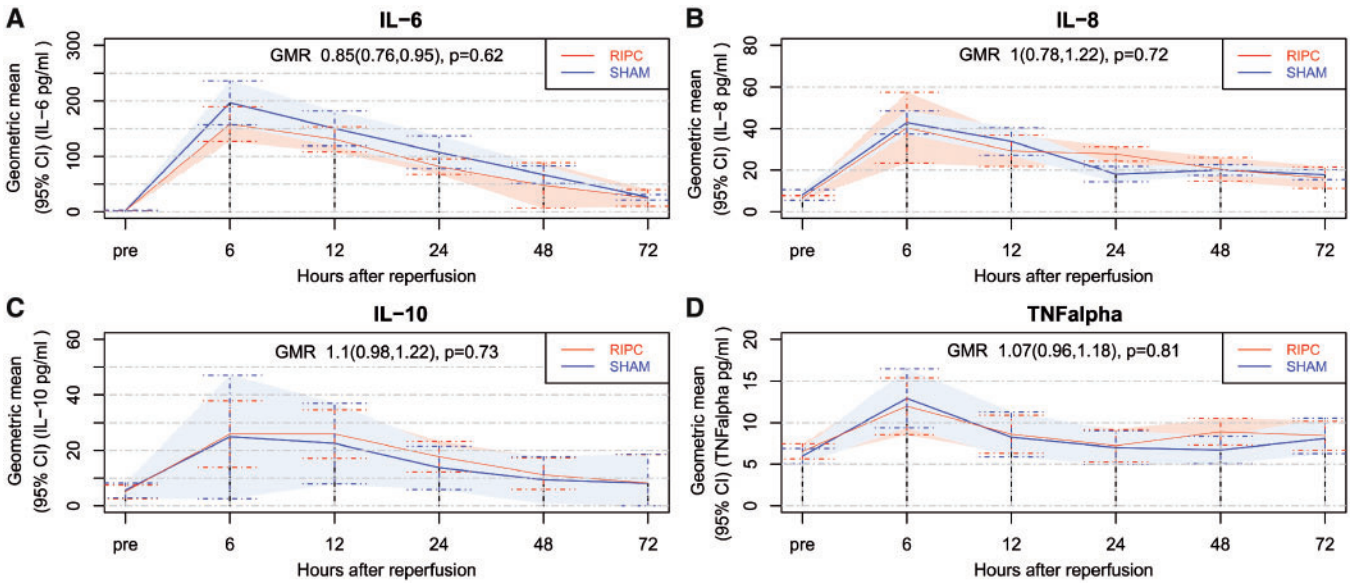

Figure 3: Coronary artery bypass grafting: concentration over time. Geometric mean and 95% CI at each study time point by group, and GMR and 95% CI for the effect of RIPC versus SHAM on (**A**–**C**) IL-6, 8, 10 and (**D**) TNF-α. CI: confidence interval; GMR: geometric mean ratio; IL: interleukin; pre: preoperative; RIPC: remote ischaemic preconditioning; TNF: tumour necrosis factor.

The coronary artery bypass grafting group. The analysis of the adenine nucleotides of the left ventricle and right ventricle biopsies are illustrated in the Supplementary Material, Fig. S2, and summarized in the Supplementary Material, Table S4. No statistical difference was observed at the level of the left and right ventricles in terms of the phosphorylation potential ($P = 0.84$, 0.76, 0.71, 0.92, ATP/ADP, ATP/AMP, the left ventricle and right ventricle RIPC versus SHAM, respectively) and the cardiomyocytes energy charge between the RIPC/SHAM groups after ischaemic reperfusion injury ($P = 0.65$, 0.88, the left ventricle and the right ventricle RIPC versus SHAM, respectively).

The aortic valve replacement group. The analysis of the adenine nucleotides of the left ventricle and right ventricle biopsies are illustrated in the Supplementary Material, Fig. S3, and

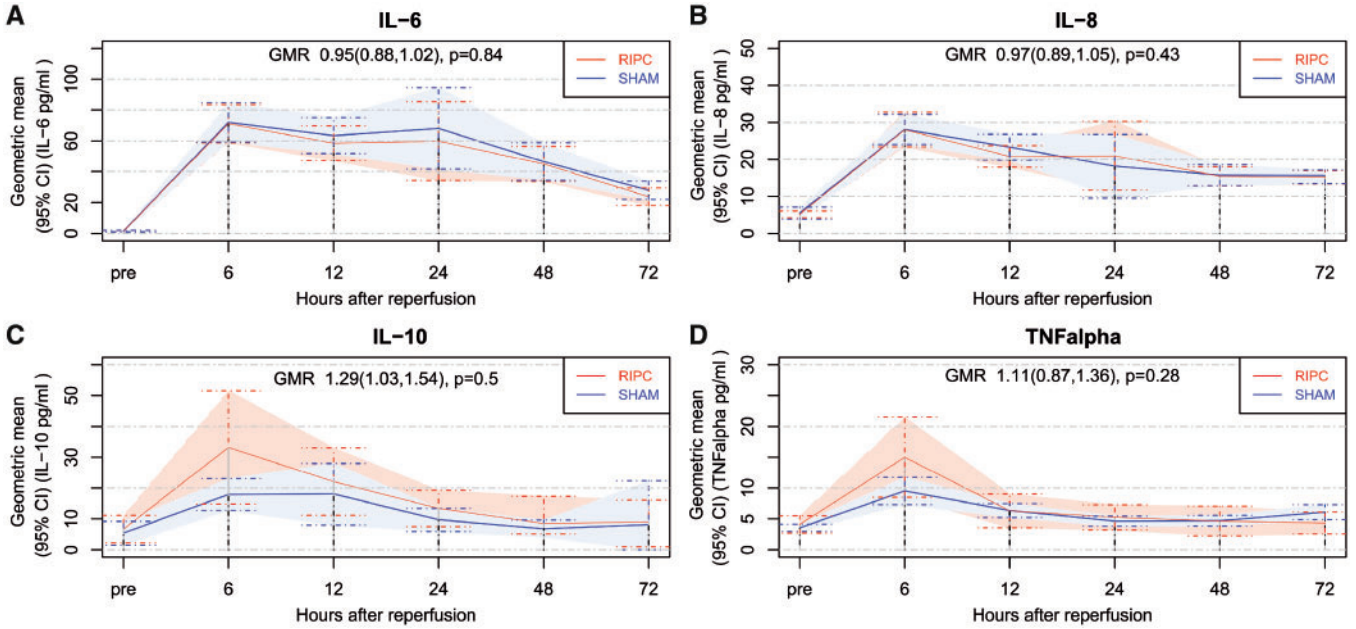

Figure 4: Aortic valve replacement: concentration over time. Geometric mean and 95% CI at each study time point by group, and GMR and 95% CI for the effect of RIPC versus SHAM on (**A**–**C**) IL-6, 8, 10 and (**D**) TNF-α. CI: confidence interval; GMR: geometric mean ratio; IL: interleukin; pre: preoperative; RIPC: remote ischaemic preconditioning; TNF: tumour necrosis factor.

summarized in the Supplementary Material, Table S4. No statistical difference was observed at the level of the left and right ventricles in terms of the phosphorylation potential ($P = 0.74$, 0.67, 0.50, 0.89, ATP/ADP, ATP/AMP, the left and right ventricle RIPC versus SHAM, respectively) and the cardiomyocytes energy charge between RIPC/SHAM groups after ischaemic reperfusion injury ($P = 0.96$, 0.78, the left ventricle and the right ventricle RIPC versus SHAM, respectively).

DISCUSSION

To the best of our knowledge, this is the first study in human that investigates the effect of RIPC on troponin, inflammatory markers and myocytes metabolites of the left and right ventricles in 2 different cardiac pathologies.

Preconditioning did not seem to confer any additional cardioprotection in terms of troponin I, inflammatory markers reduction and the left ventricle and the right ventricle energy metabolites preservation. These findings are in line with the report of the 2 largest prospective trials on RIPC in cardiac surgery [1, 2].

The ERICCA trial recruited the high-risk patients undergoing CABG ±AVR and failed to detect any benefit in the group randomized to RIPC [1]. The RIPHeart study led to the same conclusions [2]. Similar neutral findings were reported by the Remote reconditioning Trialists' Group in the most recent meta-analysis, which included 23 trials of RIPC with a total of 2200 patients undergoing cardiovascular surgery [9].

The coronary artery disease and the aortic valve disease are associated with specific disease-induced cellular remodelling, because they may exhibit the specific cellular proteome and may respond differently to ischaemic reperfusion injury [10]. Similarly, the left and right ventricles may have, as previously demonstrated, different protein profiles [9]. Different authors [11–13], upon calculating markers of ischaemic stress including the phosphorylation potential and the energy charge, found RIPC to have a significant effect. On the contrary, we previously reported that RIPC was associated with a lower phosphorylation potential after RIPC but before the ischaemic reperfusion injury compared to controls in mice [5].

Neutral results were observed in our study in both diseases in left and right ventricle biopsies, questioning the uncritical interpretation of results from experimental clinical models to the clinical scenario.

Ischaemic reperfusion injury and CPB used during cardiac surgery elicit systemic inflammatory responses that may ultimately contribute to myocardial dysfunction and postoperative complications [14]. Accordingly, it has been proposed that RIPC confers systemic protection by eliciting an anti-inflammatory response and antiapoptotic gene activation [14–16]. In our study, we did not find any significant differences in terms of pro- and anti-inflammatory cytokines in both pathologies.

There are many confounders that may undermine the effect of the RIPC [4]. Patients with coronary disease may be already 'naturally preconditioned' by a previous episode of transient ischaemia. There are evidences that both propofol and volatile (e.g. sevoflurane) anaesthetic regimes used in the heart surgery may elicit cardioprotection [17]. In the ERICCA trial, we included both anaesthetic regimens, whereas in the RIPHeart study, only propofol-based anaesthesia was used.

It may be plausible that RIPC may be associated with some harmful events [18]. We did not find any difference in the clinical outcomes, but our study was not powered to achieve this.

This study had several strengths. It was a 2-centred prospective double-blinded randomized trial that used a sham control (inflation of the cuff under the surgical drape beside the patient) to prevent surgeon or physician bias. It included 2 different

populations with specific diseases; in the AVR stratum, patients had normal coronary artery physiology with no anticipated natural preconditioning, whereas a certain degree of natural preconditioning phenomenon was expected in the CABG group due to previous angina. It also investigated the effect of RIPC in 2 different proteonomic scenarios (the coronary disease and the aortic valve disease) in left and right ventricle biopsies. Furthermore, this study has strong elements of novelty: it compares blood (troponin) and myocardial biomarkers of injury (inflammatory response, energy charge and phosphorylation potential) in humans.

Limitations

Perhaps, the biggest study limitation was the use of both propofol and volatile (sevoflurane) anaesthetic regimens during surgery. Both anaesthetic regimens can potentially interfere with preconditioning effects. There was also a certain level of heterogeneity with a large number of patients with diabetes and history of MI in the SHAM CABG group. Lastly, mid- and long-term follow-ups were not conducted in this study; however, survival after surgery has been shown to correlate with early troponin release, which was no different in both groups of our study.

CONCLUSION

In patients undergoing isolated CABG or AVR, preconditioning did not seem to confer any additional cardioprotection in terms of troponin I, inflammatory markers reduction and the left ventricle and right ventricle energy metabolites preservation.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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Conflict of interest: none declared.

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