Donagh A. Healy MSc MRCSI^{1,2}

Iain Feeley MBBS¹

Cillian J. Keogh MBBS¹

Timothy G. Scanlon MD FFRRCSF³

Philip A. Hodnett MD FFRRCS³

Austin G. Stack MD FRCPI⁴

Mary Clarke Moloney PhD⁵

Peter Whittaker PhD⁶

Stewart R. Walsh MSc FRCS²

¹Department of Surgery, University Hospital Limerick, Ireland

²Department of Surgery, National University of Ireland Galway, Galway, Ireland

³Department of Radiology, University Hospital Limerick, Limerick, Ireland

⁴Department of Medicine (Division of Nephrology), University Hospital Limerick, and Graduate Entry Medical School, University of Limerick, Limerick, Ireland

⁵Health Research Institute, University of Limerick, Limerick, Ireland

⁶Cardiovascular Research Institute and Department of Emergency Medicine, Wayne State University School of Medicine, Detroit, USA

Remote ischemic conditioning and renal function after contrast-enhanced CT scan: A randomized trial

Abstract

Purpose: Remote ischemic conditioning has been shown to protect against kidney injury in animal and human studies of ischemia-reperfusion. Recent evidence suggests that conditioning may also provide protection against kidney injury caused by contrast medium. The purpose of this study was to determine if conditioning protected against increases in serum creatinine (SCr) after contrast-enhanced computed tomography (CECT).

Methods: A randomised controlled trial (NCT 01741896) was performed with institutional review board approval and informed patient consent. Adult in-patients undergoing abdomino-pelvic CECT were allocated to conditioned or control groups. Conditioning consisted of four cycles of five minutes of cuff-induced arm ischemia with three minutes of reperfusion applied ~40 minutes before CECT. The primary outcome was SCr change after CECT.

Results: Baseline characteristics were similar in both groups. For all patients, conditioning reduced the risk ratio (RR) of increased SCr; RR 0.65 (95% confidence intervals 0.41 to 1.04). The protective effect was greater and the evidence for protection stronger when analysis was restricted to patients with pre-scan reduced renal function (eGFR <90 mL/min/1.73 m²); RR 0.40 (95% confidence intervals 0.17 to 0.95). Logistic regression revealed that conditioning was the only model variable that predicted decreased SCr; odds ratio 0.24 (95% confidence intervals 0.07 to 0.84) in patients with reduced baseline eGFR.

Conclusion: Remote conditioning decreased the risk of CECT-associated increases in serum creatinine by 60% in patients with reduced baseline eGFR. Stratification of analysis based on baseline eGFR is warranted because benefit from conditioning will occur only when there is risk of injury.

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Correspondence to:

Mr. Donagh A. Healy

Department of Surgery, University Hospital Limerick,

Dooradoyle,

Limerick, Ireland.

Email: donagh1@hotmail.com

Remote conditioning, the phenomenon whereby brief periods of ischemia-reperfusion in one tissue or organ protect against later ischemic insult in a distant tissue, was first proposed [1] and confirmed in the heart [2]. Subsequently, its application was extended to protect against kidney injury and consequent loss of function. Renal protection has been demonstrated in animal models of ischemia-reperfusion [3, 4] and in human surgical practice after abdominal aortic aneurysm repair [5, 6], coronary artery bypass grafts [7, 8], and renal transplantation [9, 10]. The concept of remote conditioning was recently further extended to reduce the incidence of contrast agent-mediated damage [11]. Contrast agents enhance diagnostic imaging but, because they are eliminated through the kidneys, can also cause injury. The mechanism of such renal injury is multifaceted and complex [12] but, because ischemia-reperfusion plays a role [13], remote conditioning might limit damage. Most studies designed to investigate this protection focus on severe kidney injury, so-called contrast-induced nephropathy (CIN), in patients with acute myocardial infarction [14-16]. Nonetheless, emerging evidence indicates that even small decreases in renal function are important because they are associated with later adverse outcomes [17]. Remote conditioning was hypothesized to reduce the incidence of such decreases; therefore, we aimed, in a randomized trial, to determine if remote conditioning protected against increases in serum creatinine (SCr) after non-emergent contrast-enhanced computed tomography (CECT) scans. Under these circumstances, we anticipated any change in SCr would be modest, but nevertheless responsive to remote conditioning.

Methods

Study population

Institutional review board approval was obtained and the single-center trial was registered (NCT01741896; <http://clinicaltrials.gov>); no changes were made after initial registration. No interim analyses were undertaken and the recruitment for the trial was stopped when 100 patients were recruited.

Eligible participants were in-patients aged over 17 years scheduled for abdomino-pelvic CECT-scans who were likely to remain in hospital for at least two days after the scan. Our exclusion criteria were as follows: allergy or hypersensitivity to iodinated contrast, hospital admission SCr $>150 \mu\text{mol/dL}$ (a contraindication to iodinated contrast), prior renal transplant, history of acute renal failure that required management by a nephrologist, and current use of either sulphonylurea or nicorandil.

Written informed consent was obtained. Patients were randomized to either remote conditioning or no intervention (1:1) using a block design (block sizes of 4, 6, and 8 were used) stratified by the presence of diabetes mellitus and chronic kidney disease (CKD; defined as baseline eGFR $<60 \text{ mL/min/1.73 m}^2$). The sequence was computer-generated by a third party not involved in the trial. Nobody else had access to the randomization sequence. Allocation concealment was achieved using sequential sealed envelopes. The envelopes were opened approximately 40 minutes before the anticipated scanning time. Three investigators (DH, IF, CK) performed all recruitment, randomization, remote conditioning, and data collection. A single investigator was available each day and if two eligible patients were scheduled for scans in close succession, only one was recruited; hence, not all consecutive eligible patients were recruited.

Protocol

Patients underwent conditioning approximately 40 minutes before contrast was given; the procedure took 32 minutes. The conditioning stimulus comprised four, five-minute cycles of arm ischemia with three minutes of reperfusion between each cycle. We induced ischemia by repeated inflation and deflation of a blood pressure cuff positioned on the patient's arm. Ischemia was achieved by inflating the cuff to a pressure of 200 mmHg or 15 mmHg above systolic pressure if that was $>200 \text{ mmHg}$. Control group patients received no sham intervention.

All patients received an intravenous bolus of iohexol (Omnipaque, GE Healthcare, Oslo, Norway), iopamidol (Niopam 300, Bracco Ltd, Buckinghamshire, UK), or iodixanol (Visipaque, GE Healthcare, Oslo, Norway). At University Hospital Limerick, most patients receive a dose of 90 mL, but patients heavier than 110 kg may receive 120 mL. All patients with eGFR $<60 \text{ mL/min/1.73 m}^2$ receive iodixanol. Any use of hydration prior to the procedure was at the discretion of the physician who ordered the scan.

The primary outcome was the change in SCr after the CECT-scan. Serum creatinine was measured using kinetic alkaline picrate methodology (Architect c Systems, Abbott Laboratories, Illinois, USA). Samples were obtained at three times; before the scan, and at 24 and 48 hours after. Secondary outcomes were serum urea at 24 and 48 hours after the CT-scan, incidence of reduced urine output (defined as $<30 \text{ mL/hour}$ for five consecutive hours) within 48 hours of the scan, and length of hospital stay from the scan until discharge date. There was no prespecified subgroup analysis; however, because contrast-related effects on kidney function are more likely to

occur in patients with already reduced renal function, we performed analysis on participants with decreased eGFR (<90 mL/min/ 1.73 m 2), as defined by the National Kidney Foundation Clinical Practice Guidelines [18].

Statistical analysis

Continuous variables were reported as means and 95% confidence intervals (CI) or medians with interquartile range (IQR) as appropriate. Intergroup comparisons were made using Student's t-test or Mann-Whitney U test. Categorical parameters were presented as proportions with their corresponding 95% CI and were compared using the Chi square test. Risk ratios (RR) were calculated for the reduction in incidence of CECT-scan-associated increases in SCr. Logistic regression was used to determine parameters associated with decreased kidney function. Formal power analysis calculations were not performed because there was no comparable study to provide guidance; however, we determined, with our proposed enrolment, a reduction of $>33\%$ in the incidence of increased SCr would be required. Analyses were performed using Stata version 12.1 (StataCorp, College Station, Texas, USA).

Results

Patients were recruited from November 2012 to March 2013: 202 patients were assessed, 102 were excluded (Figure 1) and 100 were randomized equally between groups. The primary reasons for the CECT-scans were abdominal pain (control 65%, conditioned 54%; $P=0.40$) and suspected malignancy (control 31%, conditioned 43%; $P=0.29$).

No adverse events were associated with conditioning; however, three patients failed to complete the conditioning protocol because of discomfort associated with inflation of the pressure cuff. One patient completed two cycles of ischemia-reperfusion, one completed three cycles, and one completed four cycles of three-minute inflations (because the patient was unable to tolerate five minute cycles). Time constraints prevented two additional patients receiving the complete conditioning protocol; one completed three cycles and the other one cycle. We employed intention-to-treat analysis and included these patients.

No differences were found in patient characteristics, medications, and baseline clinical parameters most likely to influence outcomes (Table 1). Similarly, the incidence of other comorbidities (smoking history, previous cardiac procedure, chronic obstructive pulmonary disease, and benign prostatic hyperplasia) and medication use (beta blocker, angiotensin receptor blocker, angiotensin converting enzyme inhibitor,

calcium antagonist, and warfarin) did not differ. Eighty three patients received 90 mL of contrast, three received 100 mL (two conditioned), and one control received 120 mL. No difference was found in nonsteroidal anti-inflammatory drug use between groups although, on day two post scan, there was weak evidence that conditioned patients had greater use (13% vs. 2%; $P=0.06$). Given the number of comparisons made, it is possible that this difference could have occurred by chance alone.

Seven patients (three conditioned), discharged before any post-scan SCr measurement, were excluded from risk ratio and logistic regression analysis. Twelve patients (eight conditioned) discharged after one post-scan SCr measurement were included. Four of these (two per group) had reduced eGFR. Because the direction of most (79%) patients' one-day SCr change corresponded to their two-day change, we do not believe this inclusion produced misclassification and bias.

Conditioning reduced the risk of increased SCr after CECT-scan. For all patients, evidence in support of a difference was weak; however, the evidence strengthened when we assessed patients with reduced baseline eGFR (Table 2). Furthermore, there was also evidence for a difference amongst conditioned patients when divided on the basis of baseline eGFR; reduced eGFR *versus* normal – RR = 0.40 (0.17 to 0.94; $P=0.02$).

Logistic regression analysis with increased SCr as the binary outcome was also conducted. When conditioning was included as a variable, there was weak evidence for a reduction in odds ratio (OR); OR = 0.45 (0.19 to 1.06; $P=0.07$). The model was not improved by addition of any other variables (evaluated using likelihood ratio tests). When analysis was restricted to patients with reduced baseline eGFR, the evidence for a protective effect of conditioning was again strengthened (OR 0.24 (0.07 to 0.84); $P=0.02$). Use of likelihood ratio tests indicated the model was not improved by addition of any other variables.

No inter-group differences were found in any of the secondary endpoints. None of the patients had reduced urine output. There were no differences in serum urea after CECT-scan at either day-one (control 3.9 [IQR 3.2, 5.5] mmol/L; conditioned 4.2 [IQR 3.2, 5.7] mmol/L; $P=0.23$) or day-two (control 4.1 [IQR 3.3, 5.6] mmol/L; conditioned 4.2 [IQR 3.1, 6.0] mmol/L; $P=0.76$). Hospital length-of-stay was also similar (control 5 [IQR 3, 9] days, conditioned 4 [IQR 2, 9] days; $P=0.59$). If analysis was restricted to patients with reduced baseline eGFR, we still did not find any differences.

FIGURE 1. Trial flow diagram

TABLE 1. Patient characteristics, comorbidities, medications, and baseline parameters

	Control (n = 44)	Conditioned (n = 43)	P value
Male	59 (44, 74)	51 (36, 66)	0.16
Age (years)	62 (57, 67)	63 (57, 69)	0.82
Body mass (kg)	83 (74, 91)	79 (73, 85)	0.44
Current smoker	16 (5, 27)	21 (8, 33)	0.55
IDDM	7 (0, 14)	5 (0, 11)	0.66
NIDDM	7 (0, 14)	12 (2, 21)	0.44
Angina pectoris	2 (0, 7)	5 (0, 11)	0.54
Hypertension	41 (26, 56)	44 (29, 59)	0.76
Antiplatelet agent use	27 (14, 41)	23 (10, 36)	0.67
Statin use	32 (18, 46)	33 (18, 47)	0.94
Diuretic use	18 (6, 30)	23 (10, 36)	0.56
Pre-hydration treatment	32 (18, 46)	42 (27, 57)	0.33
Pre-scan SCr ($\mu\text{mol/L}$)	75 (62, 85)	73 (59, 85)	0.50
Pre-scan serum urea (mmol/L)	4.3 (3.1, 5.4)	4.7 (3.8, 5.9)	0.44

Values are expressed as proportions (%) together with their 95% confidence intervals. When units are given and the distribution of the values was normal (age and body mass), the values represent means and 95% confidence intervals. When the distribution was not normal (SCr and serum urea), the values represent medians and the interquartile range.

IDDM – insulin-dependent diabetes mellitus; NIDDM – noninsulin-dependent diabetes mellitus; SCr – serum creatinine

TABLE 2. Risk of increased serum creatinine after contrast-enhanced CT-scan

Patients	N	Conditioned risk	Control risk	RR (95% CI)	P value
all	87	0.37 (16/43)	0.57 (25/44)	0.65 (0.41-1.04)	0.07
normal eGFR	40	0.55 (11/20)	0.60 (12/20)	0.92 (0.54-1.56)	0.75
reduced eGFR	47	0.22 (5/23)	0.54 (13/24)	0.40 (0.17-0.95)	0.02

Normal defined as eGFR $>90 \text{ mL/min/1.73 m}^2$, reduced eGFR defined as $<90 \text{ mL/min/1.73 m}^2$ [18]

CI – confidence interval; N – number of patients; RR – risk ratio

Discussion

Remote conditioning was found to decrease the risk of CECT-scan-associated increases in serum creatinine by 60% *versus* no intervention in patients with reduced baseline eGFR ($>90 \text{ mL/min/1.73 m}^2$).

Analysis stratification

We propose analysis stratification based on renal function is warranted because conditioning-mediated benefit occurs only

when there is risk of injury; therefore, patients with normal kidney function (i.e., large functional reserve) exposed to small volumes of contrast medium would not show ill-effects. Additional support for such stratification comes from our finding of a 60% risk reduction for serum creatinine increases in conditioned patients with reduced eGFR versus conditioned patients with normal eGFR; identical to that found in conditioned patients with reduced eGFR versus controls with reduced eGFR (Table 2).

We recommend stratification be considered in all conditioning analysis. The association between minimal risk and minimal benefit was demonstrated in cardiac studies; for example, when the area at risk of infarction was less than 25% of the left ventricle, no difference in infarct size was observed between remote conditioned and control patients with ST-segment elevation myocardial infarction (STEMI) [19]. Infarct size reduction in conditioned hearts was apparent only when risk area exceeded 25% of the left ventricle. Similar results were found by Ovize and colleagues in data from four post-conditioning studies in patients with STEMI [20]. The degree of protection depended upon the size of the risk region (assessed as a percent of ventricular circumference). Little evidence of protection was seen when risk regions were less than 35%. For large risk areas, infarcts in conditioned hearts were appreciably smaller than controls. Some renal conditioning studies avoided the minimal risk issue by enrolling only patients with CKD. Other studies, including ours, did not. Crimi and colleagues examined renal function (assessed by maximum post-procedure SCr) in remote conditioned and control patients after percutaneous intervention for STEMI and found no overall group difference [21]. Nevertheless, when they divided patients according to baseline eGFR, conditioning was associated with reduced maximum SCr for the lowest tertile (<77 mL/min/1.73 m²), but not the others (77-95 and >95 mL/min/1.73 m²). This finding is consistent with our results and emphasizes the importance of risk stratification.

Lack of stratification may explain equivocal results derived in some meta-analyses of conditioning [22, 23]. For instance, meta-analysis of remote conditioning's effect on acute kidney injury in patients undergoing vascular and cardiac procedures found only weak evidence of benefit. The combined risk ratio was 0.70 (95% CI 0.48 to 1.02; $P=0.06$) [23]. Not all included studies reported eGFR. Nonetheless, mean baseline eGFR in the conditioned group of the four most negative contributors was high and, we suggest, constituted minimal risk (85 ± 34 mL/min/1.73 m² [6], 115 (range 62 to 152) mL/min/1.73 m² [24], 82 ± 20 mL/min/1.73 m² [25], and 101 ± 20 mL/min/1.73 m² [26]). In contrast, eGFR in the most positive study was consistent with CKD and thereby provided opportunity for protection (41 ± 9 mL/min/1.73 m² [15]). Numerous factors influence outcomes after conditioning; however, we propose that risk stratification in renal studies should be routine.

Significance of small changes in kidney function

Our analysis focused on the direction of SCr change. Still, the specific amount and potential clinical significance must be con-

sidered. When SCr increases occurred in patients with reduced baseline eGFR, both groups exhibited similar changes; control 10 (95% CI 5 to 15) $\mu\text{mol/L}$ and conditioned 13 (95% CI 8 to 19) $\mu\text{mol/L}$. Such SCr increases corresponded to eGFR changes of; control -9 (95% CI -13 to -4) mL/min/1.73 m² and conditioned -14 (-22 to -6) mL/min/1.73 m². The changes are far from the magnitude seen in CIN. Nevertheless, a 10% increase in relative risk of death and non-fatal cardiovascular events was reported for each 10-unit reduction in eGFR below 81 mL/min/1.73 m² in 14,527 patients after myocardial infarction [17]. For patients with intracerebral haemorrhage, a 24% reduction in odds of death was associated with each 10 mL/min/1.73 m² increase in eGFR [27]. A retrospective study of 29,388 patients reported increased risk of CKD, progression of CKD, and even death if patients experienced an increase in serum creatinine in the first seven days after cardiac surgery [28]. These events occurred even if the increase was only 1-24%. Accumulating evidence illustrates association between reduced renal function and adverse outcomes. These range from increased mortality after stroke [29] and increased all-cause mortality [30] to increased cardiovascular events [31] and anticoagulation instability during warfarin therapy [32]. Protecting against small decreases in kidney function should provide benefit.

Medications and comorbidities

We excluded patients treated with sulphonylurea, but not those with comorbidities sometimes associated with loss of condition-mediated protection (advanced age and diabetes mellitus) [33, 34]. In a mouse model of myocardial infarction, hearts from mice with type 1 and type 2 diabetes were not protected by postconditioning [35]; hence, inclusion of subjects with diabetes mellitus could be problematic. Fifteen percent of our patients had diabetes mellitus versus 64% in a study that found remote conditioning had no effect on kidney injury after coronary bypass graft surgery [36]. This study [36] also included patients treated with sulphonylurea (21%). Moreover, their patients were approximately eight years older than ours. Comorbidities should be considered when designing future trials; nevertheless, two trials of remote conditioning and CIN, which are currently enrolling patients [37, 38], do not list diabetes mellitus as an exclusion criterion.

Limitations

Efforts to translate conditioning-mediated renoprotection from successful animal studies to clinical practice have been disappointing and obstacles remain [39,40]. The limitations of

our study illustrate some reasons for this disappointment and also some of the obstacles. First, SCr is only a proxy for kidney function and one affected by factors other than changes in function. Additionally, we do not know if the observed SCr changes persist because no long-term follow-up was undertaken. Second, few patients were found to be at risk of kidney injury after stratification and, therefore, the study was underpowered. Future studies should restrict enrolment to patients with CKD. This is especially important because of the likely weak nephrotoxicity of low-osmolar and iso-osmolar contrast media used in current practice. Third, our outcome was a biochemical rather than a clinical measure. Recent meta-analysis of cardiovascular surgery application of conditioning highlighted the importance of this difference. Remote conditioning consistently demonstrated protection when biochemical endpoints were assessed; however, when clinical outcomes were examined, there was little apparent benefit [22]. Fourth, our conditioning protocol might be suboptimal. For logistical reasons, we used three minute reperfusion periods rather than the typical five. Finally, there is evidence, based on meta-analysis of animal studies, that conditioning is more effective in kidneys when applied >24 hours before injury [41].

Conclusion

Our study provides support for the hypothesis that remote conditioning protects against contrast-mediated kidney injury. Furthermore, our study indicates the importance of risk stratification when analysing conditioning studies. Designing studies to investigate contrast-mediated kidney damage presents challenges because the injury is multifaceted. Nonetheless, conditioning represents an attractive therapy because its protection is multifaceted. Remote conditioning-mediated effects range from the first-reported protection against ischemia-reperfusion injury, to enhanced endothelial cell function [42], attenuated platelet activation [43] and platelet-mediated thrombosis [44], altered thrombus fibrin organization [45] and enhanced thrombolysis [46], and increased microvascular blood flow [47]. These could all mitigate kidney injury.

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