

# Remote Ischemic preconditioning in prevention of contrast induced nephropathy: A Randomised control trial.

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## Research article

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# Abstract

**Background:** Contrast induced nephropathy (CIN) following angiography is one of the leading causes of in-hospital acute kidney injury (AKI). The aim of this study was to investigate the renoprotective effect of remote ischemic preconditioning (RIPC) to prevent CIN in patients with peripheral arterial disease (PAD) undergoing lower limb angioplasty with standard preventative measures.

**Methods:** 40 adult patients (eGFR  $\geq$ 45 ml/min) undergoing peripheral arterial angioplasty received either: (1) Control: standard preventative measures comprising intravenous (IV) hydration with 0.9% normal saline (1ml/kg/hour) or (2) RIPC: four-5 min inflations and deflations of a pneumatic cuff placed on the upper arm in addition to IV hydration prior to the angiographic procedure. Serial measurements of serum creatinine, serum cystatin and urinary NGAL were taken at baseline and 2, 24, 48- and 72-hours post-procedure. The primary outcome was CIN as defined by a rise of creatinine by  $\geq$  26.5 $\mu$ mol/L above baseline within 48 hours of contrast exposure. Other outcome measures included the rise in urine NGAL and serum cystatin from base line at 2 hours, as an early marker of acute kidney injury (AKI).

**Results:** Both groups had similar baseline characteristics. All recruited patients had eGFR  $\geq$ 45 ml/min, and RIPC had no renoprotective effect. AKI occurred in five (13%) patients. Changes in serum creatinine at 2 hours post-procedure did not correlate with changes in urine NGAL or serum Cystatin C.

**Conclusion:** In stable PAD patients (eGFR  $\geq$ 45ml) undergoing lower limb angioplasty with standard preventative measures, RIPC did not offer any protection against development of CIN.

This study is registered on ClinicalTrials.gov (Clinical Trials No. NCT02516072)

## Background

Peripheral arterial disease (PAD) affects between 3-10% of the population with prevalence rates rising with age to 15-20% in patients over 70 years old [1]. Increasing numbers of affected patients require angiography as either a diagnostic or a therapeutic modality to improve peripheral blood flow and relieve the symptoms of chronic limb ischemia (CLI). Use of iodinated contrast media during diagnostic or therapeutic procedures can lead to contrast induced nephropathy (CIN) either by direct toxic effects on tubular cells or by induction of renal ischemia [2, 3]. CIN is an important cause of hospital acquired acute kidney injury (AKI) [3].

AKI has been defined as an acute deterioration in renal function, as defined by an increase of serum creatinine by 25% or by a factor of 0.5mg/dl over baseline within 48 hours of administration of intravenous contrast and in the absence of other causes of renal dysfunction [2,3]. Whilst the incidence of CIN in the general population is only 2%, it can be as high as 20-30% in high-risk patients. Important risk factors for the development of CIN include pre-existing chronic kidney disease, diabetes mellitus, hypertension, elderly and congestive cardiac failure [4,5,6].

A large cohort study of 5787 patients with advanced PAD found that both moderate and severe renal insufficiency was associated with increased odds of death. The 1-year mortality risk was noted to be higher in patients with severe renal insufficiency (GFR<30ml/min/1.73m<sup>2</sup>) (OR: 2.97 95%ci: 2.39-3.69) and they tended to have a higher risk of presenting with tissue damage (ischemic ulceration or gangrene) compared with individuals having normal renal function (OR: 2.21, 95% CI: 0.64-2.98) [7]. Zaraca et al. in a recent systematic review reported on the incidence of CIN as 9.2% in patients undergoing vascular surgery. The identifiable risk factors included age >70 years, high contrast volume, pre-existing renal disease and the use of antihypertensive medications [8, 9].

Remote ischaemic preconditioning (RIPC) is a protective mechanism, which was first observed in the canine heart. Brief periods of ischemia in the limbs induced using blood pressure cuffs, have been shown to confer protection from acute kidney injury following cardiac surgery or coronary angioplasty. This protective effect has been reported to extend up to 6 months following percutaneous coronary interventions [10].

This study aims to evaluate the potential of RIPC prior to vascular angiography in reducing the risk of developing contrast-induced nephropathy. The primary end point is a reduction in the prevalence of contrast medium–induced nephropathy (defined as an increase in the serum creatinine (Ser C) concentration of >25% from the baseline value within the 72-hour period after primary angiography). Secondary outcome measures include the determination of urinary NGAL levels and cystatin C levels as early biomarkers of AKI. Additional outcome measures include length of hospital stay, need for dialysis and mortality.

## Methods

**Setting:** Changi General hospital, Singapore.

### Study Population

This clinical trial (NCT02516072) was in accordance with good clinical practice and the Declaration of Helsinki [11] and ethical approval was obtained from the local Institutional review board (CIRB No. 2014/2067). This study was carried out between Jan 2015 and August 2018. Eligible subjects were adult patients (>21 years of age) with PAD scheduled for peripheral angiography or angioplasty. Exclusion criteria were severe renal impairment (eGFR <30ml/min), evidence of acute renal failure or patients on dialysis, history of previous CIN, pregnancy, and contraindications to volume replacement therapy or those on Glibenclamide or Nicorandil as these may interfere with RIPC.

Written informed consent was obtained from each patient who agreed to participate.

### Study design

All subjects received intravenous hydration via a weight-based formula (1ml/kg/hour) with 0.9 % normal saline for 6 hours prior to the administration of contrast. A web-based service was used to generate a random sequence list with block randomization method stratified to either receive RIPC or serve as control group. The study team and statistician were blinded to treatment allocation. The control patients had a “sham” RIPC with the cuff on their arm and no inflations. Patients were randomized 1:1 to receive one of the following treatments with no intention of replacement for dropouts:

Group 1 (Control): Patients received the intravenous hydration protocol only prior to the angiographic procedure

Group 2 (RIPC): Patients received the intravenous hydration protocol prior to the angiographic procedure, and RIPC, comprising placing a pneumatic cuff on the upper arm, and inflating it to 250mmHg for 5 min and deflating it for 5 min, a cycle which was repeated a total of four times. The time interval between the last cuff inflation and contrast administration were between 10-60 minutes.

All patients had serial measurements of serum creatinine as well as both urinary and serum NGAL and cystatin C, at baseline prior to the procedure and 2, 24, 48- and 72-hours post-procedure.

### **Laboratory Measurements**

All analyses were performed in the Department of Laboratory Medicine at Changi General Hospital; a College of American Pathologists accredited facility.

Serum creatinine was measured on the Cobas 6000 (Roche Diagnostics Asia-Pacific, Singapore) autoanalyzer using the kinetic Jaffe colorimetric assay. The analytical range of serum creatinine is from 15-2200 umol/L (0.17-24.9 mg/dl); inter-assay precision of creatinine is 2.5% at 100 umol/L (1.13 mg/dl) and 1.0% at 360 umol/L (4.07 mg/dl).

Serum cystatin was also measured on the Cobas 6000 using a particle-enhanced immunoturbidimetric assay. The analytical range of serum creatinine is from 0.4-6.8 mg/L; inter-assay precision of cystatin is <2.0% at 0.92 mg/L and 1.75 mg/L.

Urine NGAL was measured on the Architect i2000 analyzer (Abbott Diagnostics, Singapore) using a chemiluminescent micro particle immunoassay. The measuring range for the NGAL assay is from 10.0-1500.0 ng/mL and the inter-assay precision is <7%.

### **Statistical Analysis**

Descriptive statistics are presented as mean (SD) or median (range) for continuous variables following a Normal and non-Normal distribution respectively, and frequency and percentage for categorical variables. Fisher's Exact Test was used to assess the association between of RIPC and 25% creatinine reduction. Comparisons between the RIPC and control groups in changes in creatinine between baseline, 48 hours and 72 hours were carried out using analyses of covariance. Changes in NGAL and cystatin (which both

followed non-Gaussian distributions) were made using Mann-Whitney U-test. Associations between changes in creatinine and changes in NGAL and cystatin were assessed using Spearman correlations. All comparisons were two-sided and a p value of < 0.05 was taken as statistically significant. Statistical data analysis was performed with SPSS statistical software, version 19.0.

### Power Calculation

The sample size calculation was based on previous studies that observed that retinol binding protein (RBP), a sensitive urinary biomarker of renal damage, peaked at 699 mg/mmol, on the second post-procedure day in the control group.[12, 13]. There were no earlier studies which used rise of serum creatinine as defined by the KDIGO guidelines for diagnosis of CIN. For this study to show that RIPC would produce a 50% reduction in the urinary RBP level from 700 to 350 mg/mmol, 40 patients (half in each group) in total would be needed to give at least 80% power to show a significant difference at the 5% level.

## Results

There were 40 subjects with a baseline eGFR > 45ml/min, these patients were randomly allocated to the control group or received RIPC in a 1:1 ratio. Of the 40 patients, 39 completed the study. One patient in the control group withdrew consent, as he did not want to undergo subsequent blood sampling. The treatment groups were similar with respect to demographic and co-morbidity factors except for IHD, with 74% of RIPC patients having IHD compared to 40% of the controls (p value < 0.05?) (Table 1). 5/39 (13%) patients developed AKI after contrast exposure, 3 in control group and 2 in the RIPC group [Fisher's Exact test; p=1.00]. No patients needed dialysis. There were no adverse events noted due to the RIPC protocol.

There were no significant differences between the RIPC and control groups in the change in creatinine, NGAL or cystatin over the study period (Table 2 and Figure 2). There was no significant association between creatinine changes and changes in NGAL and cystatin at 2 hrs, 48hrs or 72 hrs (all Spearman correlations less than 0.21).

## Discussion

The increasing incidence of PVD often mandates use of contrasted procedures in the form of diagnostic angiography or angioplasty. CIN has been reported in up to 9.2% of patients undergoing peripheral angiography and contributes to increased length of hospital stay, morbidity and mortality [14].

We defined CIN as an increase in serum creatinine by  $\geq 26.5$   $\mu\text{mol/L}$  within 48 hours of contrast exposure as per the KDIGO workgroup [15].

Renal biomarkers are reported to rise earlier than serum creatinine in AKI. Serum and urine NGAL, cystatin C, urinary retinol binding protein (RBP) have all been investigated to detect AKI early. [10,16,17]. Wang et al in a recent meta-analysis, which include 1520 patients undergoing coronary angiography found urine

and plasma NGAL had a sensitivity and specificity of 84% and 89% respectively in predicting contrast induced AKI. They reported NGAL determined 4 hours after exposure to contrast was an excellent predictor of contrast-induced AKI [17].

Conversely, Moledina et al studied the performance of serum creatinine and other kidney injury biomarkers like NGAL, in a group of deceased donors who had undergone kidney biopsy at the time of organ procurement. 155/581 (27%) deceased donors had histologically proven ATN. They reported NGAL lacked accuracy in the diagnosis of acute tubular injury with an area under the receiver operating characteristic curve (AUROC) for diagnosing acute tubular injury of 0.60 (95% CI, 0.55-0.66; P=0.005) [18].

Rise in serum creatinine post-contrast exposure remains the current gold standard in the diagnosis of CIN. We did not find any correlation between rise of NGAL or Cystatin C to serum creatinine. [Table 2]

Preventative strategies for CIN include hydration with saline, isotonic bicarbonate; N-Acetylcysteine, RIPC and Xanthine have been reported earlier. There is still discrepancy in literature as to which preventative strategy is best [19]. RIPC has been demonstrated to have a protective effect against AKI in those undergoing primarily cardiac procedures like surgery or coronary interventions. [20]

A prior study evaluating the protective effects of RIPC in patients undergoing contrasted abdominal CT scan reported a 65% risk reduction in rise of serum creatinine in the group subjected to ischemic pre-conditioning [21].

Menting et al studied a group of 76 patients at risk for development of CIN as per the Dutch guidelines. The inclusion criteria were, reduced eGFR and two additional risk factors like age, heart failure, use of diuretics etc. in those undergoing contrasted studies for diagnostic or interventional purposes [22].

While they found no difference in the change of serum creatinine from baseline to 48-72 hours, in sub-analysis of the group with a high Mehran score (>11) the rise in serum creatinine was statistically lower [22].

However, there is data, which also suggests protective measures against CIN may be ineffective. A recent network meta-analysis reported on 124 trials with 28,240 patients undergoing coronary angiography, did not find either intravenous saline, isotonic bicarbonate, NGAL, xanthines or RIPC to be protective [23].

From our study of 40 patients, we did not find a significant difference in creatinine, NGAL or cystatin changes between those with and without RIPC at 48- and 72-hours post contrast exposure, and no significant correlation with change in creatinine, NGAL or cystatin from pre-op to 2 hours.

In our study population, 5 patients developed CIN, 3 from the control group and 2 from the RIPC group.

The major strengths of this study are related to its design as a blinded study with a previously registered protocol. The physicians and nursing staff were blinded to the allocation minimizing risk of bias because of adherence to preventative measures like saline infusion and the volume of contrast used.

Our study has several limitations, which may account for our findings:

1. Our sample size was based on earlier studies, which investigated urinary biomarkers to detect AKI and was not based on rise in creatinine as defined by KDIGO. Hence, our sample size may have been an underestimate. This is a common problem when biomarkers are used as a surrogate marker for AKI.
2. RIPC was added on to other protective measures like normal saline infusion and use of low osmolar contrast media. These preventative measures may have masked the development of CIN
3. Our patient cohort had eGFR >45 ml/min. This group is less susceptible to CIN as compared to those with lower eGFR. Our results may therefore represent a significant type 2 error.

The reason for inability to enroll patients with eGFR 30 to <45ml/min as per our protocol is based on our local experience, as patients chose against contrast procedures when explained the risk of CIN. We also tend to use carbon dioxide as mode of contrast under IR guidance for these patients.

Should we be targeting RIPC to those with GFR < 45 in future studies? Authors who have found protective effect of RIPC have had majority of patients with eGFR of <45 ml/min. however in our study we did not find protective effect of RIPC in patients with eGFR of >45 ml/min.

## Conclusion

We found RIPC did not offer any protective effect over and beyond saline hydration and use of low osmolar contrast in patients with eGFR >45 ml/min. The rise in Urinary NGAL and Serum Cystatin C did not correlate with rise in serum creatinine at 2 hours post contrast exposure. Since this study did not explore the possible benefits of RIPC in high-risk group (eGFR < 45 ml/min) further studies should be pursued to look at RIPC in the high-risk group of developing CIN.

## Declarations

Ethics approval and consent to participate: Singhealth IRB (CIRB NO: 2014/2067). Written informed consent.

Consent for publication: not applicable.

Availability of data and material: the dataset used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interest: all the authors declare that they have no competing interest.

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Role of the funding body in design of the study, collection, analysis and interpretation of data and in writing the manuscript: none.

Authors Contributions: DR, PSD, TTY, ATC, HDJ, WSR contributed equally to the design, collection of data and writing the paper. JM carried out the statistical analysis

All authors have read and approved the final manuscript.

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Study status: trial has been completed.

## Abbreviations

contrast induced nephropathy (CIN), acute kidney injury (AKI), intravenous (IV), peripheral arterial disease (PAD), retinol binding protein (RBP).

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## Tables

**Table 1:** Demographic factors and co-morbidities by randomised group

	Mean (range) or % (number)		
	Overall cohort (n=39)	Control (n=20)	RIPC (n=19)
Age (yrs.)	65.7 (42,89)	65.4 (52, 84)	66.0 (42, 89)
BMI	24.7 (14.7,39.8)	24.7 (14.7, 39.8)	24.7 (18.1, 37.0)
Systolic BP	142 (100,194)	136 (100, 176)	148 (125, 194)
Diastolic BP	73.3 (50,90)	73.5 (61, 90)	73.2 (50, 90)
DM	90% (35)	95% (19)	84% (16)
PVD	100% (39)	100% (20)	100% (19)
IHD	56% (22)	40% (8)	74% (14)
CVA/TIA	23% (9)	25% (5)	21% (4)
Hypertension	97% (38)	100% (20)	95% (18)
Anaemia	5% (2)	0% (0)	10% (2)
COPD	5% (2)	5% (1)	5% (1)
Malignancy	3% (1)	5% (1)	0% (0)

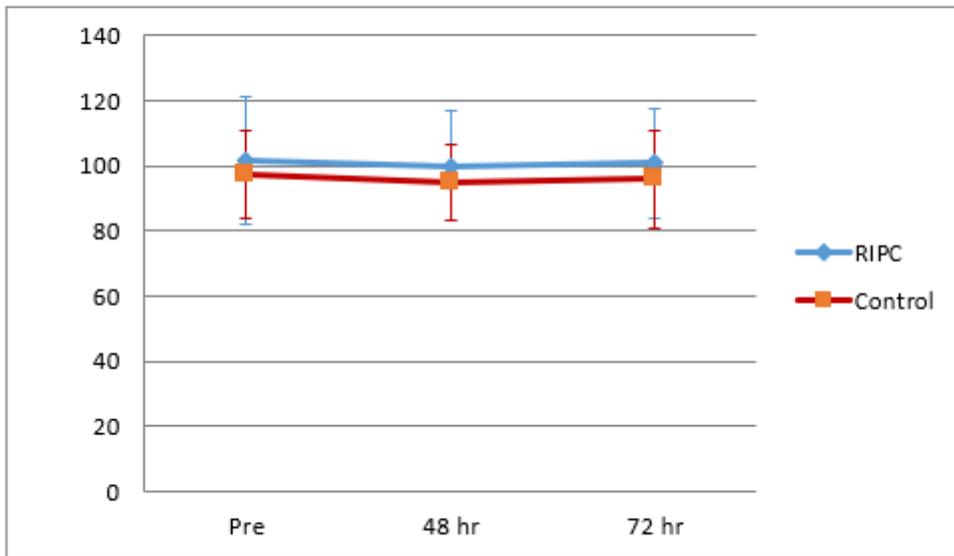
**Table 2:** Change in Serum creatinine, NGAL and Cystatin C in the RIPC and Control group between baseline and at 48 hours and 72 hours.

	Mean (95% CI)		Comparison
	Control	RIPC	control vs RIPC
Serum Creatinine			
48 hrs. - baseline	-2.7 (-8.7, 3.3)	-1.0 (-7.0, 5.0)	P=0.69
72 hrs. - baseline	-0.4 (-7.6, 6.7)	-0.3 (-7.5, 6.9)	P=0.98
NGAL <sup>1</sup>			
48 hrs. - baseline	4.0 (-5.2, 28.7)	-3.0 (-9.4, 82.7)	P=0.71
72 hrs. - baseline	28.8 (-13.0, 48.7)	8.7 (-7.9, 37.4)	P=0.96
Cystatin <sup>1</sup>			
48 hrs. - baseline	-0.06 (-0.14, 0.06)	-0.06 (-0.24, -0.02)	P=0.36
72 hrs. - baseline	-0.005 (-0.12, 0.06)	-0.01 (-0.26, 0.03)	P=0.31

<sup>1</sup> Median values

## Figures

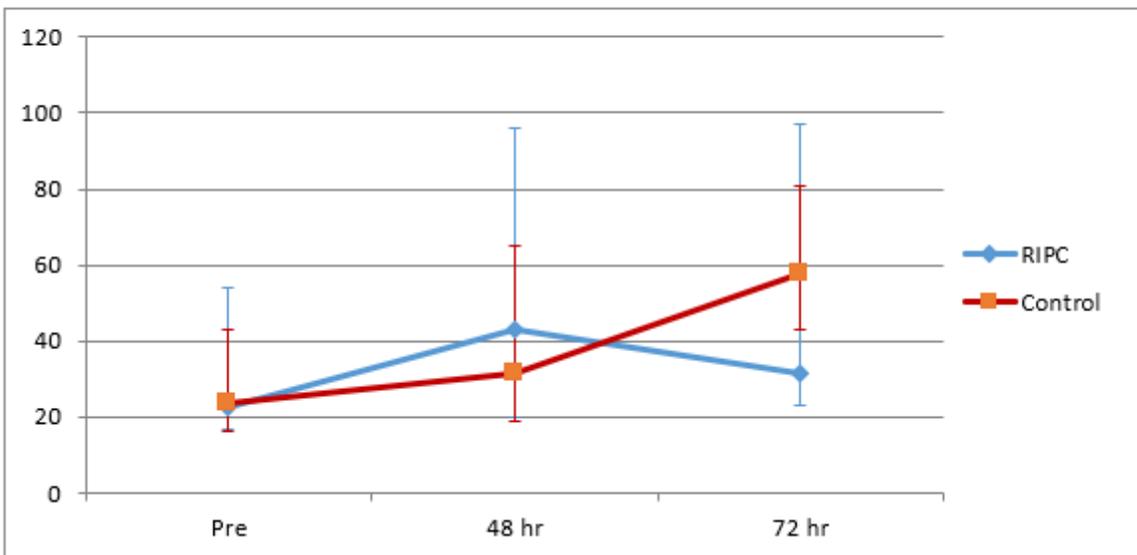
## Serum Creatinine



**Figure 1**

Changes in serum creatinine over the study period. X-axis: time(hours), Y-axis: serum creatinine [mean value(umol/L)]

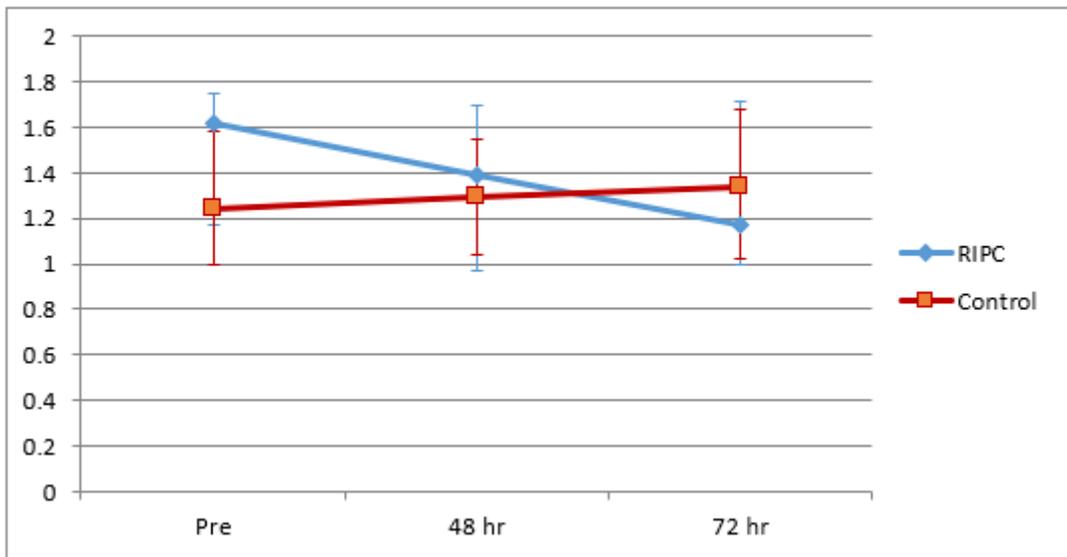
## Urinary NGAL



**Figure 2**

Urinary NGAL change over study period. X-axis: time(hours), Y-axis: urine NGAL [median value (mg/ml)]

## Serum Cystatin



**Figure 3**

serum Cystatin C over study period. X-axis: time(hours), Y-axis: serum Cystatin C [median value(mg/L)].

## Supplementary Files

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