Author’s response to reviews

**Title:** Remote ischemic preconditioning to reduce contrast-induced nephropathy: study protocol for a randomized controlled trial

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**Author’s response to reviews:** see over
Point-by-point responses to the reviewer’s comments on the manuscript

Remote ischaemic preconditioning to reduce contrast-induced nephropathy: study protocol for a randomized controlled trial

We thank the reviewers for their comments and the Editorial board for the careful evaluation and for giving us the opportunity to revise our manuscript. Here we provide a point-by-point response to the comments of all reviewers.

Editorial requests

1) Please ensure the title conforms to journal style for study protocol articles. The title should follow the format ‘____________: study protocol for a randomized controlled trial’?

Response: We apologize for this error and changed the title: ‘Remote ischaemic preconditioning to reduce contrast-induced nephropathy: study protocol for a randomized controlled trial’ (page 1, lines 2, 3).

2) Please ensure that your trial status sections includes a description of where you were up to when you submitted the manuscript.

Response: At the time of submission 61 patients were included in the study. We added this information to the ‘trial status’ section (page 11, line 6).

3) If applicable, please include an acknowledgement section at the end of the manuscript before the reference list. Please acknowledge anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include the source(s) of funding for all authors. Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements. Please state clearly whether or not you have funding in the acknowledgement section. If there is no funding, please state this.

Response: This trial is partially funded by a research grant from COOK Medical. We added this information to the acknowledgement section (page 11, line 27).

4) Please mention each author individually in your Authors Contributions section. We suggest the following kind of format (please use initials to refer to each author’s contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript?

Response: We changed the authors contribution section into: TS performed (sham) ischaemic preconditioning, participated in the collection of data and drafted the manuscript. TM performed (sham) ischaemic preconditioning and participated in the collection of data. YW performed (sham) ischaemic preconditioning and participated in the collection of data. RD participated in the design of the study and performed the statistical analysis. KW participated in the design of the study. SL
participated in the coordination of the trial. DV participated in the coordination of the trial. JW participated in the design of the study. LS participated in the design of the study. MW participated in the design of the study and its coordination and helped to draft the manuscript. All authors revised the manuscript and gave I approval of the final version.’ (page 11, lines 16-24).

5) Please include a figure title and legend section after the reference list. The figures should not be included in the main body of the manuscript.

Response: We apologize for including the figure in the main body. The figure title has been added after the reference list (page 14, line 34). In our view a figure legend would not be necessary.

Reviewer 1

1) Introduction section – first paragraph – it would be of relevant if the authors were able to provide some discussion on the biologic rationale and mechanisms by which RIPC may mediated AKI in general and after ischemia/contrast exposure.

Response: We thank the reviewer for the suggestion to provide some additional information on the biologic rationale and mechanism by which RIPC may reduce ischaemia-reperfusion injury of the kidney (whether or not induced by contrast administration). The following text has been added to the introduction section: ‘Remote ischaemic preconditioning (RIPC) is a short and harmless discontinuation of blood supply to particular organs or tissue, followed by reperfusion. A preconditioning stimulus is applied before the onset of prolonged ischaemia. In animal models it has been found to reduce ischaemia-reperfusion injury of the kidney [25]. Although the precise mechanism of RIPC remains unknown, two major pathways may play a pivotal role: the humoral and neurogenic pathway. Both are thought to induce various kinase cascades and eventually prevent opening of the mitochondrial permeability transition pore in the target organ, thereby reducing cell death.’ (page 3, lines 24-30).

2. Introduction section – first paragraph – the authors should provide more specific rational and justification for essentially replicating the pilot Renal Protection Trial by Er et al published in Circulation. What are the key elements on the Renal Protection Trial that predispose it to bias and justify this study?

Response: We fully agree with the reviewer that this is a highly important issue that should be better addressed. As discussed by in a letter by Mehta Oza, the reported mean Mehran risk score of 13 by Er et al. would provide a 26% to 30% incidence of CIN based on Mehran’s development and validation data sets. Er et al. reported a 40% incidence of CIN and therefore the question arises whether standard measures to prevent CIN, i.e. hydration with saline and discontinuation of diuretics, were carried out appropriately. Data on compliance to those standard preventive measures were not provided by Er et al. This important issue was also not addressed by Er et al. in their discussion. In fact, the high incidence of CIN was attributed to the high prevalence of heart failure and diabetes mellitus in their cohort. However, the Renal Protection Trial was adequately blinded and therefore the risk of bias is low. The sentence in the discussion section (page 10, lines 4, 5) “this study may have been exposed to a certain degree of selection bias” is therefore deleted.

Nevertheless, the RenPro trial has two major limitations: 1) no information was given on the degree of adherence to standard measures to prevent CIN, 2) a cohort of patients with an unusual high risk of CIN was selected. As these limitations confine the generalizability of the RenPro trial, we propose a
study in which all patients at risk of CIN according to the Dutch guideline will be included. Point 2) is the main reason to perform our study and this is emphasized in the introduction section (page 4, lines 8, 9, 11, 12). Point 1) has been added to the discussion section (page 10, lines 10-14).

How will this proposed smaller trial better clarify the question of whether RIPC is efficacious for prevention CIN?

Response: First, we aim to design a (proof-of-concept) study with a better external validity / generalizability (as discussed above; introduction section page 4, lines 11, 12). We agree with the reviewer that a large clinical trial would be required to assess whether or not RIPC reduces the incidence of CIN. However, in our view the change in serum creatinine level from baseline to 48-72 hours after contrast-administration is an appropriate primary endpoint. Considering the definition of CIN is based upon this change in serum creatinine. Obviously, the change in serum creatinine as primary end-point allows the design of a study with a smaller number of patients.

3. Methods section – third paragraph - the authors propose to also include patients receiving both intravenous and intra-arterial contrast. Are the differences in the risk of CIN associated with these routes of delivery of contrast that will a priori be evaluated?

Response: We agree with the reviewer that this is an important issue to address. To maximize external validity of our study, we did not exclude patients receiving intravenous contrast. To our knowledge, there are no studies reporting a significant difference in contrast-induced kidney injury between patients receiving intravenous versus intra-arterial contrast. In our previous work, we also did not observe a significant difference between these routes of delivery (Balemans et al., ref. 17).

4. Methods section – while the author intent to enroll high risk patients – perhaps the integration of a clinical risk score (Mehran risk score) similar to the Renal Protection Trial (Er F et al Circulation 2012) would help benchmark the risk between studies and enable better comparison?

Response: We fully agree with the reviewer that the Mehran risk score is required to relate the results of our study to others. Er et al. included patients with low (<5) as well as high (>16) Mehran risk scores. To maximize external validity of our study, the Mehran risk score will not be used for exclusion of patients in this study. To assess whether RIPC has a different impact in patients with high and low Mehran scores, a subgroup analysis will be performed. For this analysis patients will be divided according to their Mehran risk score (added to the ‘statistical analysis’ section; page 8, lines 26-29).

5. Methods section – Primary Endpoint - time frame for ascertainment of changes in SCr – I would suggest the authors have a concrete definition for their protocol (i.e., 72 hours) and not a range of 2-3 days (i.e., 48-72 hours) – as there is inherent risk patients may develop the outcome of interest on day three yet be missed. The trial registration website only states 48 hours – not 72 hours.

Response: First we apologize for the discrepancy with the trial registration website! We agree with the reviewer that it would be ideal to have 72 hours (or perhaps 48 AND 72 hours) as time-point for serum creatinine measurement, however –in our experience- a significant number of the patients has difficulties to (re)visit their general practitioner or clinic for blood sampling at a fixed day. Furthermore, the Dutch guideline prescribes to check renal function within 48-72 hours after contrast-administration. To optimize the feasibility of the study, we allowed the range of 48-72 hours. We
addressed this limitation in the discussion section (page 10, lines 22-28). Also we will request to change the information at the registration website.

6. Methods section – Adverse events – the authors should specifically list and define the most commonly anticipated AE expected from the trial.

Response: To our knowledge, serious adverse events due to the use of RIPC with an extremity as remote organ are extremely rare. Mild adverse events, related to the compression of an inflated blood pressure cuff around the upper arm, might be: transient discomfort, hematoma formation (i.e. ecchymosis) at the upper arm or petechiae at the lower arm. This is added to the methods section (page 7, lines 27-29).

7. Methods section – Power calculation – can the authors justify why they expect RIPC to reduce serum creatinine further below baseline by 14 mcmol/L? Is this expected to be a clinically meaningful change in SCr that correlated with patient-centred events – in particular when a key trial outcome (though secondary) is categorical (CIN - yes/no)?

Response: We agree with the reviewer that the choice for a further reduction in serum creatinine of 14 µmol/L should be clarified. In the study by Er et al., change in serum creatinine from baseline to 48 hours was 0.41mg/dL in the control group and 0.16 mg/dL in the experimental group. A difference of 0.25 mg/dL between these values could be attributed to the use of RIPC. A difference of 14 µmol/L (0.158 mg/dL) corresponds with approximately 60% of the effect that was found in the Renal Protection trial. As this is still a large effect, it would not be appropriate to speak of a 'clinically meaningful difference'. To address this issue, we added to the ‘power calculation’ section, that a difference of 14 µmol/L between the control and experimental group corresponds with approximately 60% of the mean difference that was found in the Renal Protection trial by Er et al. (page 8, lines 10-11).

8. Methods section – Statistical analysis – this section would benefit from expansion to clarify the primary and secondary analyses proposed.

Response: Primary, univariable analyses will be performed to detect differences between the control and experimental arm with regard to: baseline characteristics and primary and secondary outcome measures. Secondary, a multivariable analysis will be performed only with regard to the primary outcome measure. The secondary analysis also includes a subgroup analysis to assess the influence of the Mehran risk score. The has been clarified in the methods, statistical analysis section (page 8, lines 21-29).

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

9. Study title – this should include “Study Protocol for a Randomized Control Trial” as per journal standards.

Response: The title has been changed according the reviewers’ suggestion (page 1).

10. Introduction section – first paragraph – it is uncertain what the authors are referring to when they described “mortality ratio” – is this simply the observed crude mortality? This should be clarified
– and perhaps a relative risk of death instead compared with those not developing CIN would also be of benefit to cite.

Response: Indeed it is the observed mortality during the index hospitalization. We changed this in the introduction section (page 3, lines 10, 11).

11. Methods section – the trial registration website states biomarkers will be captured from blood/urine at 24 hours after contrast exposure.

Response: Biomarker analysis at 4-6 hours after contrast exposure will be performed to assess early markers of CIN. We will request to change ‘24 hours’ to ‘4-6 hours’ at the registration website.

In addition – the secondary outcome of CIN on the trial registration website is stated as a 25% increase in SCr only – and not a 25% or 0.5 mg/dL change.

Response: We thank the reviewer for noticing this discrepancy. Indeed we defined CIN as a 25% increase in SCR and/or 0.5 mg/dL change. We will request to change the information at the registration website.

In addition – the trial registration website states the trial is COMPLETED. These are not discussed in the protocol and inconsistent with the registration website.

Response: Currently, the trial is completed. At the time of submission of this manuscript, 61 patients were included.

12. Introduction section – first paragraph – “ischaemia”, “pathophysiology” and “the” are spelled incorrectly.

Response: We sincerely apologize for these errors.

Reviewer 2

1. The authors have planned to conduct this RCT with a relatively small of number of patients. This small n affects the definition of the primary outcome, as the standard definition of CIN could not be applied, i.e. an increase in creatinine levels by 25% or 0.5 mg/dl within 48-72 hours. This is a significant limitation of the study which needs to be clearly stated.

Response: We fully agree with the reviewer and we clearly stated in the discussion section that change in serum creatinine levels are appropriate for proof-of-concept studies. Much larger clinical trials are required to confirm the results with regard to clinically more relevant endpoints such as dialysis and/or death (page 10, lines 28-30 and page 11, lines 1-3).

2. The authors have defined the primary outcome as a change in serum creatinine levels from baseline to 48 to 72 hours. The sample size is calculated on the assumption that fluid administration decreases creatinine levels and that RIPC can further decrease creatinine levels. The concept that fluid administration decreases creatinine levels needs to be clarified. This concept has been detailed in some references such as Moran and Myers, Kidney Int 1985; Pickering Crit Care 2013. It is counterintuitive to have at a decrease in creatinine levels as the primary outcome in a study on CIN.
A larger recent randomized controlled study (225 patients) on the same subject including patients undergoing urgent percutaneous coronary intervention found that serum creatinine increased by 14% in the control group and by 7% in the RPIC group, which is not concordant with the power calculation presented in this study (Deftereos JACC 2013). Although the authors referenced Er et al in their power calculation, this group also found similar results, i.e. an increase in serum creatinine levels in both groups at 48 hours (Er et al, Circulation 2012).

Response: The reviewer addresses an important issue. The study by Deftereos et al. investigate the effect of remote ischemic postconditioning in patients undergoing urgent PCI. As also described in the methods section by Deftereos et al., hydration protocols were only applied when possible. Furthermore, the mean baseline eGFR was 75 ml/min/1.73m². Therefore it is likely to assume that pre- and posthydration protocols were not applied in a significant part of patients enrolled in this study. This may be a plausible explanation for the fact the serum creatinine levels did not decrease in the control group. As already discussed above (point 2, reviewer 1) there are also reasons to assume that hydration protocols were not adequately applied in the study by Er et al. Finally, it is important to emphasize that we identified patients at risk of CIN according to the Dutch guideline ‘contrast-nephropathy’. Both, Deftereos and Er used different criteria for the selection of patients at risk of CIN. To maximize external validity of our study, we chose to adopt the criteria from the Dutch guideline to identify patients at risk of CIN. In the abstract, introduction and methods section we clarified that criteria from the Dutch guideline are used for the identification of patients at risk of CIN (page 2, line 20; page 4, line 13; page 5, lines 10-13, 30; page 7, line 5).

3. In their power calculation, the authors mentioned that the expected rate of loss to follow-up is 8%. However this does not seem to be taken into account in the final calculation (38 patients in the experimental and control arms, numbers that are not increased by 8%).

Response: We apologize for the confusion on this point. Our power calculation indicated that 34 patients were needed in both the experimental and control arm of the study. 4 patients were added to control for 5% (not 8%) loss to follow-up (e.g. blood sampling not performed between 48-72 hours after contrast administration). We corrected this in the methods section (page 8, lines 12, 18).

4. Why did the authors choose to ask patients to fill up a questionnaire for comorbidities and medications? Will they use chart review?

Response: We agree with the reviewer that this should be clarified. We used both questionnaires and chart review to obtain accurate and updated information with regard to comorbidities and (discontinuation of) medication. We added this to the methods section (page 7, lines 2, 3).

5. Did the authors ensure 1) to withhold nephrotoxic drugs 48 hours before and after the procedure in both groups and 2) to have a similar rate of utilization of N-acetylcysteine between groups (although its efficacy is controversial)?

Response: 1) According to the Dutch guideline, all nephrotoxic drugs were discontinued at least 24 hours before and after contrast administration. Compliance to this will be recorded by the questionnaire and double checked by the researcher (page 7, line 2). 2) N-acetylcysteine will not be used as preventive measure (as there is no evidence to do so).
Minor issues not for publication

6. The authors should include references to justify the cut-off of >100 mL intravascular contrast (McCullough PA, Am J Med 1997, Manske, Am J Med, 1990)

Response: We thank the reviewer for suggesting two relevant references. We added them to the methods section to clarify the cut-off value of >100 mL intravascular contrast (page 5, lines 15-17).

7. There are many typos in the text which should be corrected, such as:
a. Page 3. pathophysiology
b. Page 3. Te instead of the
c. Page 5. during 5 minutes followed by 5 minutes of reperfusion T (no period at the end of the sentence)

Response: We sincerely apologize for these errors.