Original Article

Access this article online
Quick Response Code:

Website: http://www.braincirculation.org
DOI: 10.4103/bc.bc_26_18

Effect of remote ischemic preconditioning on cerebral vasospasm and biomarkers of cerebral ischemia in aneurysmal subarachnoid hemorrhage (ERVAS): A protocol for a randomized, controlled pilot trial

RP Sangeetha, VJ Ramesh, Sriganesh Kamath, Rita Christopher[1], Dhananjaya I Bhat[2], HR Arvinda[3], Dhritiman Chakrabarti

Abstract:

INTRODUCTION: Cerebral vasospasm is a dreaded complication of aneurysmal subarachnoid hemorrhage (aSAH) predisposing to delayed cerebral ischemia. We intend to study the cerebroprotective effects of remote ischemic preconditioning (RIPC) in patients with aSAH.

MATERIALS AND METHODS: This is a single-center, prospective, parallel group, randomized, pilot trial, approved by the Institutional Ethics Committee. Patients with aSAH admitted to our hospital for surgical clipping; fulfilling the trial inclusion criteria will be randomized to true RIPC ($n = 12$) (inflating upper extremity blood pressure cuff thrice for 5 min to 30 mmHg above systolic blood pressure) or sham RIPC ($n = 12$) (inflating blood pressure cuff thrice for 5 min to 30 mmHg) in 1:1 allocation ratio using a computerized random allocation sequence and block randomization.

RESULTS: Our primary outcome measure is vasospasm on cerebral angiography and transcranial Doppler study, and concentration of serum S100B and neuron-specific enolase at 24 h after RIPC and on day 7 of ictus. Our secondary outcomes are safety of RIPC, cerebral oxygen saturation, and Glasgow coma score, and extended Glasgow outcome scale scores at discharge and at 1, 3, and 6 months following discharge. Outcome measures will be assessed by an observer blinded to the study intervention.

CONCLUSION: If our preliminary results demonstrate a beneficial effect of RIPC, this would serve as a clinically applicable and safe preemptive method of protection against cerebral ischemia.

Keywords:

Biomarkers of cerebral ischemia, cerebral oxygen saturation, cerebral vasospasm, delayed cerebral ischemia, ischemic preconditioning, transcranial Doppler

Departments of Neuroanaesthesia and Neurocritical Care, [1]Neurochemistry, [2]Neurosurgery, [3]Neuroimaging and Interventional Radiology, National Institute of Mental Health and Neurosciences, Bengaluru, Karnataka, India

Address for correspondence:
Dr. Sriganesh Kamath, Department of Neuroanaesthesia and Neurocritical Care, Third Floor, Neurosciences Faculty Block, National Institute of Mental Health and Neurosciences, Bengaluru - 560 029, Karnataka, India.
E-mail: drsri23@gmail.com

Submission: 01-11-2018
Revised: 16-12-2018
Accepted: 14-01-2019

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a life-threatening disease with grave consequences. Significant complications such as cerebral vasospasm occur in 40%–70% of survivors mostly between 4 and 10 days after aSAH.[1] Vasospasm can predispose to cerebral hypoperfusion either remote to or near the site of ruptured aneurysm and correlates with extravasated blood products.[2] Although majority of ischemic lesions remain asymptomatic, they may contribute to cognitive deficits even after

How to cite this article: Sangeetha RP, Ramesh VJ, Kamath S, Christopher R, Bhat DI, Arvinda HR, *et al*. Effect of remote ischemic preconditioning on cerebral vasospasm and biomarkers of cerebral ischemia in aneurysmal subarachnoid hemorrhage (ERVAS): A protocol for a randomized, controlled pilot trial. Brain Circ 2019;5:12-8.

definitive treatment of ruptured aneurysm.[3] Exploration of safe, minimally invasive, controllable, and cost-effective neuroprotective technique during aneurysm treatment is desirable to improve cerebral ischemic tolerance.[4-7] Ischemic preconditioning has shown to protect tissue/organ from subsequent lethal injury by administering short spells of subthreshold ischemia, including during neurosurgical or neuroradiological interventions.[8,9]

Direct ischemic preconditioning using brief period of parent vessel occlusion during aneurysm surgery has shown to attenuate tissue hypoxia as measured by cerebral dialysis and tissue oxygen tension.[10]

Remote ischemic preconditioning (RIPC) involves brief episodes of nonlethal ischemia on the limbs remote from the target organ which results in release of protective factors that acts on distant organ preventing cell death,[11,12] although the exact mechanism remains to be elucidated.[13-16]

Randomized control trials have reported the efficacy of RIPC in stroke prevention in intracranial atherosclerosis[17] and carotid endarterectomy[18] with preservation of short-term postoperative cognitive function in cardiac surgical patients undergoing cardiopulmonary bypass[19] and improvement in local and systemic endothelial function and microcirculation in healthy volunteers.[20]

Prior clinical studies have demonstrated the safety[21,22] of RIPC in patients with aSAH and its potential to produce neurovascular and cerebral metabolic changes[17] and hence produce a positive effect in their functional outcomes.[23] Reduced middle cerebral artery (MCA) mean velocities, lactate/pyruvate ratios, and glycerol levels, demonstrating cerebrovascular vasodilatory and cell membrane preservation effects lasting up to 2 days following RIPC, were noted previously.[24]

To the best of our knowledge, there are no previous studies that have assessed the role of RIPC in the prevention of vasospasm in aSAH patients; hence, we propose to study the same.

Transcranial Doppler (TCD) has been used for the diagnosis of cerebral vasospasm and for predicting neurological outcome following SAH.[25]

RIPC at 6 and 24 h of hospital admission has shown to decrease S100beta (S100B) and neuron-specific enolase (NSE) and contribute to improved outcome in patients undergoing surgery for cervical spine injury[26] and traumatic brain injury.[27] Following aneurysmal SAH, elevated blood levels of both S100B and NSE have been associated with unfavorable clinical outcomes.[28]

The current study is designed to study the effects of RIPC on cerebral hemodynamics, cerebral oxygenation, serum biomarkers of cerebral ischemia, and long-term functional outcome in patients with aSAH.

Materials and Methods

Study setting

The trial will be conducted at our hospital which is an academic tertiary care neurosciences center.

Trial design

This is a prospective, randomized, controlled, parallel group, single-center pilot trial.

Ethical approval

The study is approved by our Institute Ethics Committee.

Patient recruitment

All patients who will be admitted to our hospital with aSAH without preexisting vasospasm on diagnostic angiography will be assessed by an interdisciplinary team consisting of neurosurgeons, neuroradiologists, and neuroanesthesiologists and considered for study inclusion if surgical treatment of aneurysm is decided. The primary investigator will then be informed for possible enrollment.

Blinding

Patient, physician, outcome assessor, and data analyst will be blinded to the study intervention.

Study participants

All patients with diagnosis of aSAH who are scheduled for surgical clipping will be screened for possible inclusion into the study. Informed consent will be obtained from the patients where possible and where patient's neurological status prohibits obtaining of consent from the patient; it will be obtained from the patient's relative.

Our inclusion criteria are as follows:

1. Ruptured anterior cerebral circulation aneurysm with subarachnoid hemorrhage
2. Age between 18 and 65 years
3. WFNS Grade 1 or 2
4. Presentation within 3 days of ictus
5. Consent for surgical management of ruptured aneurysm
6. Consent for participation in the study.

We will exclude patients if they fulfill the following criteria:

1. Upper limb cellulitis, ulcers, or peripheral vascular disease
2. Posterior circulation aneurysm
3. WFNS Grade ≥ 3
4. Presentation to hospital beyond 3 days of ictus

5. Inadequate transtemporal window for TCD study
6. Refusal of consent
7. Endovascular coiling or conservative management of ruptured aneurysm
8. Unruptured aneurysm.

Randomization

Block randomization with a block size of six will be done by a coinvestigator using a computer-generated random number sequencing. Patients who provide written informed consent will be randomized in a 1:1 allocation ratio to receive either true RIPC (Group A) (n = 12) or sham RIPC (Group B) (n = 12). Randomization code for each patient will be revealed only to the research assistant assigned to perform all sessions of RIPC.

Allocation concealment

Allocation concealment will be done to prevent selection bias by the investigators using a centralized service to avoid knowledge about allocation sequence in advance.

Conduct of study

Baseline assessment

Cerebral blood flow velocities in the anterior circulation will be assessed through the transtemporal bone window using TCD. Regional cerebral oxygen saturation (rScO2) will be assessed using near-infrared spectroscopy technique (NIRS) from frontally placed sensors. Blood sample will be collected for biomarkers (S100B and NSE). All baseline assessments will be performed before the study intervention (first session of true/sham RIPC). Hemodynamic parameters such as pulse rate and blood pressure will be recorded prior to, during, and after each session of RIPC and TCD examination.

Remote ischemic preconditioning and sham preconditioning

The true RIPC protocol includes three 5-min cycles of upper-limb ischemia with 5-min intervals of reperfusion in between. The cuff is inflated to a pressure 30 mmHg above the systolic blood pressure.[24] The control group (sham RIPC) receives sham preconditioning with blood pressure cuff inflated to 30 mmHg for 5 min followed by 5 min of deflation. Three such cycles are administered over a period of 30 min. The procedure will be terminated if the patient reports any discomfort in the upper limb. During RIPC, the limb is evaluated clinically for signs of ischemic damage throughout the study and documented in a checklist. Each patient receives RIPC sessions every 48 h, from the day of recruitment until 7–10 days after ictus or until discharge, whichever is earlier.

Serum biomarker analysis

Blood samples for assessment of biomarkers (S100B and NSE) will be drawn by the nursing staff at prespecified time points. Baseline sample will be collected before the first session of RIPC. The second blood sample will be collected at 24–36 h after the first session of RIPC. Venous blood (5 ml) will be collected in gel tubes, serum separated, centrifuged at 2500 revolutions per minute for 10 min, and stored at 80°C at the metabolic laboratory of Neurobiology Research Centre in our hospital.

Serum S100B and NSE will be measured by enzyme immunoassay technique based on monoclonal antibodies, specifically targeting the astrocyte-specific β-chain of the S100 dimer and by detecting the γ subunit of NSE, respectively, using commercially available kits and control materials, by the coinvestigator from the department of neurochemistry.

Aneurysm treatment

Treatment of intracranial aneurysms will consist of microsurgical clipping under general anesthesia following standard protocol.

Perprocedural data collection during surgical clipping will involve recording of the following events whenever the event occurs:

1. Intraoperative aneurysmal rupture
2. Duration of hypotension
3. Inotropes/vasopressors requirement
4. Details of temporary clipping: duration, number of times of application
5. Details of cerebral protection provided during temporary clipping
6. New-onset deficits after clipping of aneurysm

Postprocedural dose of intra-arterial nimodipine and any addition of milrinone to the treatment protocol will be noted.

Patient's condition during the hospital stay will be monitored using hemodynamic parameters, Glasgow coma scale (GCS) score, cerebral blood flow velocities using TCD, rScO2, and cerebral angiography (if indicated). If the patient is intubated, routine intensive care and monitoring will be adhered to.

Outcome assessment

This will be done by the principal investigator as follows:

Primary outcomes

1. Vasospasm as assessed on TCD and/or cerebral angiogram
   a. TCD will be done every 24 h until 7–10 days after ictus or until discharge whichever is earlier
   b. Cerebral angiography will be done at the time of diagnosis, and subsequently, for confirmation of clinically suspected vasospasm during the hospital stay

c. Clinical signs of vasospasm will also be noted.

2. Biomarkers – serum concentration of S100B and NSE levels
   a. Baseline at the time of recruitment (prefirst RIPC)
   b. At 24–36 h after the first session of RIPC
   c. Repeated at days 7–10 after ictus or at discharge, whichever is earlier.

Secondary outcomes

1. Neurological status – Change in GCS during the hospital stay and Glasgow outcome scale-extended (GOSE) score at discharge
2. The $rScO_2$ will be monitored every 24 h for the assessment of cerebral hypoxia
3. GOSE scores at 1, 3, and 6 months after discharge through a telephonic interview.

Criteria for diagnosis of vasospasm on TCD is as follows: a 2-MHz probe will be used for transtemporal insonation and bilateral MCA, anterior cerebral artery (ACA), and extracranial internal carotid artery (ICA) flow velocities will be assessed.

The following TCD parameters will be recorded:
1. Mean flow velocities of bilateral MCA, ACA, and ICA
2. Pulsatility indices of bilateral MCA, ACA, and ICA
3. Bilateral Lindegaard ratio (LR)

Vasospasm is defined by the presence of the following:
1. Mean flow velocity of MCA (Vm) >120 cm/s with LR >3[29]
2. Mean flow velocity of ACA (Vm) >120 cm/s.[29]

Criteria for diagnosis of vasospasm of any (proximal/distal) vessel on cerebral angiography[30] will be as follows:
- Grade 0 = no narrowing
- Grade 1 = slight narrowing (<25% reduction in lumen diameter)
- Grade 2 = moderate narrowing (25%–50% stenosis or 50%–75% stenosis affecting only a short segment of the vessel)
- Grade 3 = severe narrowing (50%–75% stenosis affecting a long segment of the vessel or any stenosis >75%).

Cerebral desaturation monitored using NIRS is defined as a 20% drop in $rScO_2$ compared to baseline levels on any day across the measurement period on either side.[31]

The ERVAS trial methodology is depicted in a flowchart [Figure 1].

Sample size

As this is a pilot study, sample size is not based on hypothesis testing. Based on the feasibility considerations,

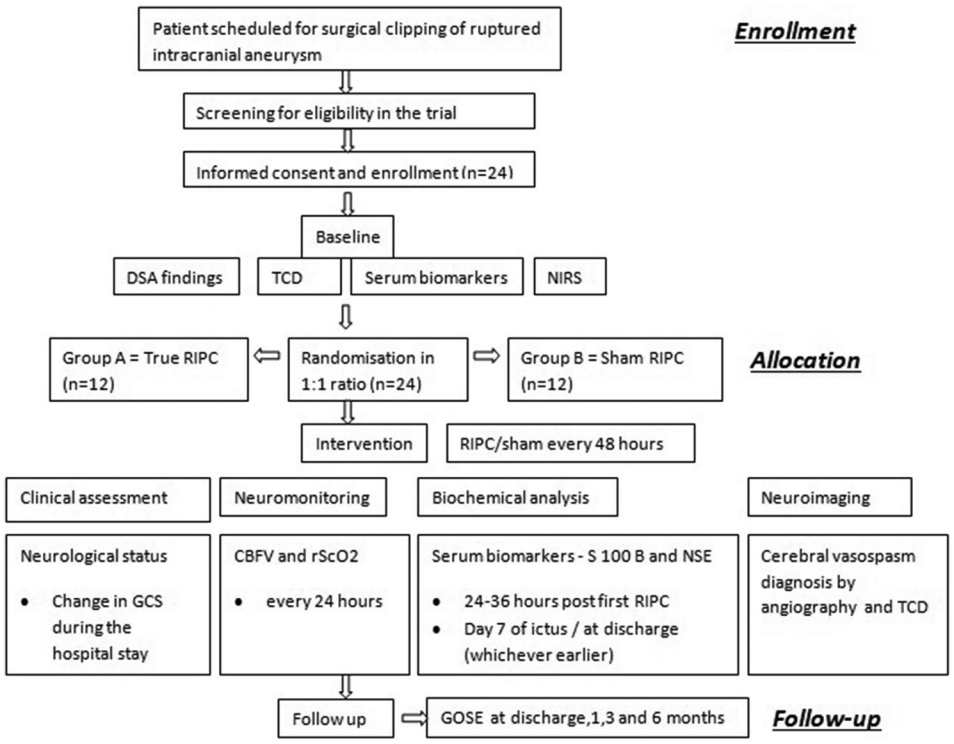

Figure 1: Flowchart of the ERVAS trial methodology. DSA: Digital subtraction angiography, S100B: calcium-binding protein S100beta, NSE: neuron-specific enolase, RIPC: remote ischemic preconditioning, TCD: transcranial Doppler, NIRS: near-infrared spectroscopy, CBFV: Cerebral blood flow velocity, rScO2: Regional cerebral oxygen saturation, GCS: Glasgow coma scale, GOSE: Glasgow outcome scale extended

we plan to include 12 patients/group suggested as appropriate in the literature for pilot studies.[32,33]

Statistical analysis

Statistical data analysis will be performed by an independent statistician using SPSS version software for Windows (SPSS Inc., Chicago, IL, USA) and R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria).[34] Values will be tabulated and mean and standard deviation will be calculated. Normality testing for quantitative variables will be done using Shapiro–Wilk test. Based on the normality, between-group testing for single variables will be done using either independent samples *t*-test or Mann–Whitney *U*-test. For quantitative data across time points, normally distributed data will be tested using repeated-measures ANOVA within -group and mixed-models ANOVA for between-group testing. For repeated-measures data, between- and within-group comparison will be conducted using rank-based factorial methods using "nparLD" package of R (Noguchi K *et al*, 2012).[35] Qualitative data will be tested between groups using Chi-square test or Fisher's exact test as appropriate and within group using McNemar's test. $P < 0.05$ will be considered statistically significant.

Ethics and dissemination

The study protocol and the process of obtaining informed consent are consistent with the Helsinki declaration.[36]

Our study is approved by the Institutional Ethics Committee on January 16, 2018 (No. NIMH/DO/ETHICS sub-committee [BS and NS DIV.] 8th meeting/2017). Informed written consent from potential trial participants will be obtained by one of the investigators before the patient recruitment in the trial.

The final results on completion of the study will be communicated to the scientific community through conference presentations and scientific publications in a peer-reviewed biomedical journal.

Discussion

This study will explore the cerebroprotective effects of RIPC in the context of aSAH. The study evaluates cerebroprotective effect of RIPC in terms of vasospasm as assessed by changes in the cerebral blood flow velocities on TCD study and cerebral angiography and biomarkers of cerebral ischemia as assessed by serum S100B and NSE. We expect RIPC to protect from cerebral vasospasm and ischemia.

Cerebral perfusion influences cerebral oxygenation, and both are pivotal for neuronal survival after an ischemic insult. Hence, we propose to monitor them together using TCD for cerebral perfusion and $rScO_2$ for oxygenation. The effect of these vital parameters on long-term functional outcomes will be evaluated.

Amid the multitude of biomarkers that have been formerly investigated for their prognostic relevance in aSAH, a combination of serum biomarkers of both astrocytic and neuronal injury – S100B and NSE, respectively, are chosen as surrogate measure of cerebral ischemia, considering their wide availability and previous evidence in cerebrovascular pathologies. There is no definite threshold established for serum levels of these biomarkers to prognosticate outcomes after ischemic injury. Through this study, we plan to explore if such a threshold can be predicted for determining neurological outcomes.

Association between cerebral vasospasm and unruptured aneurysms is rare.[37] Aneurysmal rupture with subsequent presence of subarachnoid extravasated blood predisposes these patients to vasospasm and subsequent consequences including cerebral ischemia. Hence, aSAH provides a good model to test whether RIPC helps prevent the development of cerebral vasospasm and ischemia.

Safety of remote ischemic preconditioning

The safety profile of RIPC was evaluated in prior feasibility trials which enrolled patients with aSAH[21,22] and intracranial atherosclerosis.[18,38] No patients reported ischemic pain, bruising, deep venous thrombosis, or other neurovascular injuries in these studies. However, a predefined checklist to monitor the cardiorespiratory parameters (pulse rate, blood pressure, and peripheral arterial oxygen saturation) and to assess adverse effects (pain/tenderness, paresthesia, limb weakness, bruising, erythema, and limb edema) during RIPC will be maintained for all patients for each session of true/sham RIPC.

Strengths of this study

Here, we pursue a simple, clinically applicable preconditioning technique that is successfully used in clinical trials evaluating other end-organ ischemia. If found effective, this may find application in various other areas of neurosciences.

This study explores RIPC in a highly standardized clinical situation which is aSAH with homogeneity of the study population in terms of site of intracranial aneurysm, time since ictus, general condition at admission, and the definitive treatment modality. By restricting the inclusion of patients to those presenting within 72 h of ictus, the preconditioning stimulus and day of ictus are attempted to be time-locked in a standardized fashion.

Multimodal neurological assessment of outcomes using clinical, imaging, and biochemical parameters with a long-term follow-up until 6 months after discharge is another strength of this study.

Limitations of this study

This is a single-center study. Ischemia-inducing events during surgical clipping such as brain tissue retraction, temporary cross-clipping, or occlusion of an afferent vessel for proximal control, accidental occlusion of efferent vessels, thrombosis, or thromboembolism during treatment may lead to cerebral ischemia in up to 60% of patients.[39] This can influence vasospasm and levels of serum biomarkers of cerebral ischemia.

Although our protocol for RIPC to upper extremity is predefined, there is no clarity on the ideal site, timing, duration, frequency, or indicators for adequacy of the preconditioning stimulus.

Trial status

The first patient was enrolled in January 2018. At the time of manuscript submission, enrollment of participants continues.

Acknowledgment

We are thankful to the Indian Council of Medical Research (ICMR) and the Indian Society of Neuroanaesthesia and Neurocritical Care (ISNACC) for grants for this study. External funding for this trial is sanctioned and the grant stands partially executed.

Financial support and sponsorship

The ERVAS trial is an academic trial supported by the ICMR and the ISNACC. The sponsors have no role in study design, data collection, data interpretation, or dissemination of data.

Conflicts of interest

There are no conflicts of interest.

References

1. Sharma BS, Sawarkar DP. Vasospasm: The enigma of subarachnoid hemorrhage. Neurol India 2015;63:483-5.
2. Rowland MJ, Hadjipavlou G, Kelly M, Westbrook J, Pattinson KT. Delayed cerebral ischaemia after subarachnoid haemorrhage: Looking beyond vasospasm. Br J Anaesth 2012;109:315-29.
3. Kang DH, Hwang YH, Kim YS, Bae GY, Lee SJ. Cognitive outcome and clinically silent thromboembolic events after coiling of asymptomatic unruptured intracranial aneurysms. Neurosurgery 2013;72:638-45.
4. Ginsberg MD. Neuroprotection for ischemic stroke: Past, present and future. Neuropharmacology 2008;55:363-89.
5. Klein KU, Engelhard K. Perioperative neuroprotection. Best Pract Res Clin Anaesthesiol 2010;24:535-49.
6. Kim GY. Cerebral protection. In: Vacanti C, Segal S, Sikka P, Urman R, editors. Essential clinical anaesthesia. Cambridge University Press; 2011. p. 585-90.
7. Kim YW, Zipfel GJ, Ogilvy CS, Pricola KL, Welch BG, Shakir N, *et al.* Preconditioning effect on cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. Neurosurgery 2014;74:351-8.
8. Steiger HJ, Hänggi D. Ischaemic preconditioning of the brain, mechanisms and applications. Acta Neurochir (Wien) 2007;149:1-0.
9. Dirnagl U, Becker K, Meisel A. Preconditioning and tolerance against cerebral ischaemia: From experimental strategies to clinical use. Lancet Neurol 2009;8:398-412.
10. Chan MT, Boet R, Ng SC, Poon WS, Gin T. Effect of ischemic preconditioning on brain tissue gases and pH during temporary cerebral artery occlusion. Acta Neurochir Suppl 2005;95:93-6.
11. Hausenloy DJ, Yellon DM. Remote ischaemic preconditioning: Underlying mechanisms and clinical application. Cardiovasc Res 2008;79:377-86.
12. Romera C, Hurtado O, Mallolas J, Pereira MP, Morales JR, Romera A, *et al.* Ischemic preconditioning reveals that GLT1/EAAT2 glutamate transporter is a novel PPARgamma target gene involved in neuroprotection. J Cereb Blood Flow Metab 2007;27:1327-38.
13. Kirino T. Ischemic tolerance. J Cereb Blood Flow Metab 2002;22:1283-96.
14. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124-36.
15. Stevens SL, Vartanian KB, Stenzel-Poore MP. Reprogramming the response to stroke by preconditioning. Stroke 2014;45:2527-31.
16. Narayanan SV, Dave KR, Perez-Pinzon MA. Ischemic preconditioning and clinical scenarios. Curr Opin Neurol 2013;26:1-7.
17. Meng R, Asmaro K, Meng L, Liu Y, Ma C, Xi C, *et al.* Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis. Neurology 2012;79:1853-61.
18. Walsh SR, Nouraei SA, Tang TY, Sadat U, Carpenter RH, Gaunt ME, *et al.* Remote ischemic preconditioning for cerebral and cardiac protection during carotid endarterectomy: Results from a pilot randomized clinical trial. Vasc Endovascular Surg 2010;44:434-9.
19. Hudetz JA, Patterson KM, Iqbal Z, Gandhi SD, Pagel PS. Remote ischemic preconditioning prevents deterioration of short-term postoperative cognitive function after cardiac surgery using cardiopulmonary bypass: Results of a pilot investigation. J Cardiothorac Vasc Anesth 2015;29:382-8.
20. Jones H, Hopkins N, Bailey TG, Green DJ, Cable NT, Thijssen DH, *et al.* Seven-day remote ischemic preconditioning improves local and systemic endothelial function and microcirculation in healthy humans. Am J Hypertens 2014;27:918-25.
21. Koch S, Katsnelson M, Dong C, Perez-Pinzon M. Remote ischemic limb preconditioning after subarachnoid hemorrhage: A phase Ib study of safety and feasibility. Stroke 2011;42:1387-91.
22. Gonzalez NR, Connolly M, Dusick JR, Bhakta H, Vespa P. Phase I clinical trial for the feasibility and safety of remote ischemic conditioning for aneurysmal subarachnoid hemorrhage. Neurosurgery 2014;75:590-8.
23. Laiwalla AN, Ooi YC, Liou R, Gonzalez NR. Matched cohort analysis of the effects of limb remote ischemic conditioning in patients with aneurysmal subarachnoid hemorrhage. Transl Stroke Res 2016;7:42-8.
24. Gonzalez NR, Hamilton R, Bilgin-Freiert A, Dusick J, Vespa P, Hu X, *et al.* Cerebral hemodynamic and metabolic effects of remote ischemic preconditioning in patients with subarachnoid hemorrhage. Acta Neurochir Suppl 2013;115:193-8.
25. Naqvi J, Yap KH, Ahmad G, Ghosh J. Transcranial doppler ultrasound: A review of the physical principles and major applications in critical care. Int J Vasc Med 2013;2013:629378.
26. Hu S, Dong HL, Li YZ, Luo ZJ, Sun L, Yang QZ, *et al.* Effects of remote ischemic preconditioning on biochemical markers and neurologic outcomes in patients undergoing elective cervical decompression surgery: A prospective randomized controlled trial. J Neurosurg Anesthesiol 2010;22:46-52.
27. Joseph B, Pandit V, Zangbar B, Kulvatunyou N, Khalil M, Tang A, *et al.* Secondary brain injury in trauma patients: The effects

of remote ischemic conditioning. J Trauma Acute Care Surg 2015;78:698-703.

28. Oertel M, Schumacher U, McArthur DL, Kästner S, Böker DK. S-100B and NSE: Markers of initial impact of subarachnoid haemorrhage and their relation to vasospasm and outcome. J Clin Neurosci 2006;13:834-40.
29. Bacigaluppi S, Zona G, Secci F, Spena G, Mavilio N, Brusa G, *et al.* Diagnosis of cerebral vasospasm and risk of delayed cerebral ischemia related to aneurysmal subarachnoid haemorrhage: An overview of available tools. Neurosurg Rev 2015;38:603-18.
30. Lindegaard KF, Nornes H, Bakke SJ, Sorteberg W, Nakstad P. Cerebral vasospasm diagnosis by means of angiography and blood velocity measurements. Acta Neurochir (Wien) 1989;100:12-24.
31. Negargar S, Mahmoudpour A, Taheri R, Sanaie S. The relationship between cerebral oxygen saturation changes and post operative neurologic complications in patients undergoing cardiac surgery. Pak J Med Sci 2007;23:380-5.
32. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. Pharm Stat 2005;4:287-91.
33. Hertzog MA. Considerations in determining sample size for pilot studies. Res Nurs Health 2008;31:180-91.
34. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; 2018. Available from: https://www.r-project.org/" \t "_blank" https://www.R-project.org/. [Last accessed on 2018 Aug 25].
35. Kimihiro Noguchi, Yulia R. Gel, Edgar Brunner, Frank Konietschke. nparLD: An R Software Package for the Nonparametric Analysis of Longitudinal Data in Factorial Experiments. Journal of Statistical Software 2012;50:1-23. Available from: http://www.jstatsoft.org/v50/i12/. [Last accessed on 2018 Aug 25].
36. World Medical Association declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA 2000;284:3043-5.
37. Paolini S, Kanaan Y, Wagenbach A, Fraser K, Lanzino G. Cerebral vasospasm in patients with unruptured intracranial aneurysms. Acta Neurochir (Wien) 2005;147:1181-8.
38. Li S, Ma C, Shao G, Esmail F, Hua Y, Jia L, *et al.* Safety and feasibility of remote limb ischemic preconditioning in patients with unilateral middle cerebral artery stenosis and healthy volunteers. Cell Transplant 2015;24:1901-11.
39. Tülü S, Mulino M, Pinggera D, Luger M, Würtinger P, Grams A, *et al.* Remote ischemic preconditioning in the prevention of ischemic brain damage during intracranial aneurysm treatment (RIPAT): Study protocol for a randomized controlled trial. Trials 2015;16:594.