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

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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

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...
definitive treatment of ruptured aneurysm. Exploration of safe, minimally invasive, controllable, and cost-effective neuroprotective technique during aneurysm treatment is desirable to improve cerebral ischemic tolerance. 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.

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.

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, although the exact mechanism remains to be elucidated.

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

Prior clinical studies have demonstrated the safety of RIPC in patients with aSAH and its potential to produce neurovascular and cerebral metabolic changes and hence produce a positive effect in their functional outcomes. 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.

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.

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 and traumatic brain injury. Following aneurysmal SAH, elevated blood levels of both S100B and NSE have been associated with unfavorable clinical outcomes.

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 \((\text{rScO}_2)\) will be assessed using near-infrared spectroscopy technique (NIRS) from frontally placed sensors. Blood sample will be collected for biomarkers \((\text{S100B and NSE})\). 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.\(^{[34]}\) 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 \((\text{S100B and NSE})\) 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 \((5 \text{ ml})\) 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 \(\beta\)-chain of the S100 dimer and by detecting the \(\gamma\) 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, \(\text{rScO}_2\), 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,
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 rScO₂ 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.[18,30] 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. 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.

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Conflicts of interest
There are no conflicts of interest.

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