Original Article

Access this article online

Quick Response Code:

Website: http://www.braincirculation.org

DOI: 10.4103/bc.bc_13_21

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

R. P. Sangeetha, Ramesh J. Venkatapura, Sriganesh Kamath, Rita Christopher[1], Dhananjaya Ishwar Bhat[2], H. R. Arvinda[3], Dhritiman Chakrabarti

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

Address for correspondence:
Dr. R. P. Sangeetha, Department of Neuroanesthesia and Neurocritical Care, Third floor, Neurosciences Faculty Block, National Institute of Mental Health and Neurosciences, Bengaluru - 560 029, Karnataka, India.
E-mail: sangeetharp14@gmail.com

Submission: 27-01-2021
Revised: 07-04-2021
Accepted: 15-04-2021
Published: 29-05-2021

Abstract

BACKGROUND: Cerebral vasospasm can complicate aneurysmal subarachnoid hemorrhage (aSAH), contributing to cerebral ischemia. We explored the role of remote ischemic preconditioning (RIPC) in reducing cerebral vasospasm and ischemia and improving outcomes after aSAH.

MATERIALS AND METHODS: Patients with ruptured cerebral aneurysm undergoing surgical clipping and meeting the trial criteria were randomized to true RIPC ($n = 13$) (inflating upper extremity blood pressure cuff thrice to 30 mmHg above systolic pressure for 5 min) or sham RIPC ($n = 12$) (inflating blood pressure cuff thrice to 30 mmHg for 5 min) after ethical approval. A blinded observer assessed outcome measures-cerebral vasospasm and biomarkers of cerebral ischemia. We also evaluated the feasibility and safety of RIPC in aSAH and Glasgow Outcome Scale-Extended (GOSE).

RESULTS: Angiographic vasospasm was seen in 9/13 (69%) patients; 1/4 patients (25%) in true RIPC group, and 8/9 patients (89%) in sham RIPC group ($P = 0.05$). Vasospasm on transcranial Doppler study was diagnosed in 5/25 (20%) patients and 1/13 patients (7.7%) in true RIPC and 4/12 patients (33.3%) in sham RIPC group, ($P = 0.16$). There was no difference in S100B and neuron-specific enolase (NSE) levels over various time-points within groups ($P = 0.32$ and 0.49 for S100B, $P = 0.66$ and 0.17 for NSE in true and sham groups, respectively) and between groups ($P = 0.56$ for S100B and $P = 0.31$ for NSE). Higher GOSE scores were observed with true RIPC ($P = 0.009$) unlike sham RIPC ($P = 0.847$) over 6-month follow-up with significant between group difference ($P = 0.003$). No side effects were seen with RIPC.

CONCLUSIONS: RIPC is feasible and safe in patients with aSAH and results in a lower incidence of vasospasm and better functional outcome.

Keywords:

Biomarkers of cerebral ischemia, cerebral vasospasm, delayed cerebral ischemia, Glasgow outcome scale extended, remote ischemic preconditioning, transcranial Doppler

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Sangeetha RP, Venkatapura RJ, Kamath S, Christopher R, Bhat DI, Arvinda HR, *et al*. Effect of remote ischemic preconditioning on cerebral vasospasm, biomarkers of cerebral ischemia, and functional outcomes in aneurysmal subarachnoid hemorrhage (ERVAS): A randomized controlled pilot trial. Brain Circ 2021;7:104-10.

© 2021 Brain Circulation | Published by Wolters Kluwer Health – Medknow

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is an unfavorable critical manifestation of ruptured intracranial aneurysm with deleterious/devastating consequences. About 30% of survivors of initial aSAH have a predisposition to delayed cerebral ischemia (DCI).[1] Cerebral vasospasm is one of the mechanisms for DCI after aSAH. Vasospasm is seen in about 40%–70% of patients, usually between 4 and 10 days after aSAH.[2] This is associated with high mortality and long-term dependence.[3] Interventions aimed at preventing or minimizing vasospasm are therefore likely to improve the outcomes after aSAH. The delay in the occurrence of vasospasm provides an opportunity to institute measures for its prevention. However, trials evaluating pharmacological interventions such as clazosentan, simvastatin, tirilazad, and magnesium sulfate did not find beneficial effects in preventing cerebral infarction or improving outcomes.[4]

Remote ischemic preconditioning (RIPC) is a nonpharmacological intervention wherein brief periods of nonfatal ischemia are applied to a tissue distant from the target tissue to confer protection from subsequent ischemia injury.[5] Although RIPC has been extensively studied in cardiac settings,[6] its role in neurological pathologies is explored mainly in the last decade. Initial reports in patients with aSAH have been encouraging regarding feasibility and safety,[7] cerebral vasodilatation, cell membrane preservation,[8] DNA methylation, and gene expression changes.[9] One recent clinical study has demonstrated improved functional outcomes with RIPC in patients with aSAH.[10] However, clinical trials comprehensively evaluating the effect of RIPC on vasospasm, ischemia, and functional outcomes in patients with aSAH are lacking.

In this study, we hypothesized that RIPC might have a protective role in preventing cerebral vasospasm and consequent ischemic injury, thus improving neurological outcomes in patients with aSAH. Our study's primary objective was to assess the effect of RIPC on cerebral vasospasm and biomarkers of cerebral ischemia, while our secondary objectives were to assess its impact on short-term and long-term clinical outcomes and safety in patients with aSAH.

Materials and Methods

Study setting and participants

This prospective, randomized, controlled, parallel-group, single-center pilot trial was conducted from January 2018 to November 2018 after approval from the Ethics Committee of the National Institute of Mental Health and Neurosciences, Bengaluru (No. NIMH/DO/ETHICS sub-committee [BS and NS DIV.] 8th meeting/2017), and registration with the Clinical Trial Registry of India (CTRI/2017/06/008724). The detailed study methodology is published earlier.[11] In brief, consenting adult patients with aSAH scheduled for surgical clipping of the ruptured aneurysm during the study period were recruited based on the predefined patient selection criteria.[11]

As this was a pilot study, the sample size calculation was not based on hypothesis testing. Based on the consideration of feasibility, we studied 25 patients with 12 patients in one group and 13 in another, as suggested appropriate in the literature for pilot studies.[12]

Randomization with a block size of six was done by an independent investigator using a computer-generated random number sequencing in a 1:1 allocation ratio to receive either true RIPC or sham RIPC. The patient, physician, outcome assessor, and data analyst were blinded to the study interventions.

Data collection

Baseline demographic and clinical data were noted for all patients. In addition, data regarding heart rate and blood pressure during intervention, mean cerebral blood flow velocity (mCBFV), pulsatility index (PI), and Lindegaard ratio (LR) with Trans Cranial Doppler (TCD) every day through the trans-temporal window in the anterior cerebral artery (ACA), middle cerebral artery (MCA) and internal carotid artery (ICA), and angiographic vasospasm were collected. Hemodynamic parameters were monitored before, during, and after each session of RIPC and TCD examination. Duration of hospital stay and mortality data were also collected.

Study interventions

The technique of administration of RIPC (true/sham) was as described in the published study protocol,[11] which, in brief, included inflating upper extremity blood pressure cuff thrice to 30 mmHg above systolic blood pressure for 5 min in the true RIPC group and inflating blood pressure cuff thrice to 30 mmHg for 5 min in the sham RIPC group. All patients received designated RIPC sessions every 48 h from the day of recruitment till 7–10 days after ictus or until discharge, whichever was earlier. Monitoring of hemodynamic parameters and surveillance for RIPC-associated complications, namely erythema, bruising, pain, paresthesia, limb weakness, or limb edema, if any, were documented in a predefined checklist.

Outcome assessment

Cerebral vasospasm was assessed using neurological assessment, TCD, and cerebral angiography. The CBFV was assessed through the trans-temporal TCD window in all

patients daily. Cerebral angiography was performed only in patients with a clinical suspicion of cerebral vasospasm.

Serum biomarkers of cerebral ischemia (S100B and NSE) were assessed at three time-points-before study intervention (first session of true/sham RIPC), at 24–36 h after first RIPC, and on day 7–10 of ictus. The details of diagnosis of vasospasm and cerebral ischemia are published earlier in our protocol.[11]

Neurological outcome was assessed using the Glasgow Coma Scale (GCS) score at admission and discharge and Glasgow Outcome Scale Score (GOSE) at discharge and 1, 3, and 6 months of follow-up.

Statistical analyses

Normality testing for quantitative variables was done using the Shapiro–Wilk test. Between-group testing for single variables was done using Mann–Whitney U-test. For repeated-measures data, between-and within-group comparison was conducted using rank-based factorial methods using the "nparLD" package of R.[13] Qualitative data were tested between groups using the Chi-square test and within-group using McNemar's test. $P < 0.05$ was considered statistically significant. Statistical data analysis was performed by an independent statistician using R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria).[14]

Results

A total of 90 patients admitted to NIMHANS with aSAH during the study period were screened for possible inclusion in the study. Among them, only 31 were eligible for recruitment fulfilling the predefined inclusion criteria, of which 25 patients completed all the study interventions as per randomization, 13 in the true RIPC group and 12 in the sham RIPC group. The flow of patients into the trial is depicted in the CONSORT flow diagram [Figure 1]. Baseline demographic parameters were similar in both groups [Table 1]. There was no difference in the distribution of aneurysm locations between the groups ($P = 0.581$). Anterior communicating artery aneurysms were the most common diagnosis in both groups. Among the patients who had a comorbid illness, three patients were hypothyroid (2 in true RIPC and 1 in sham RIPC group). One patient in the sham RIPC group was asthmatic. Eight were hypertensive (4 in true RIPC and 4 in sham RIPC group). One patient in the sham RIPC group had Type 2 diabetes mellitus, and two in true RIPC had a prior history of ischemic heart disease with postcardiac bypass and percutaneous coronary angioplasty status, respectively.

Cerebral vasospasm

The baseline TCD parameters (mCBFV, PI, and LR) were similar in both groups. Based on the MCA flow

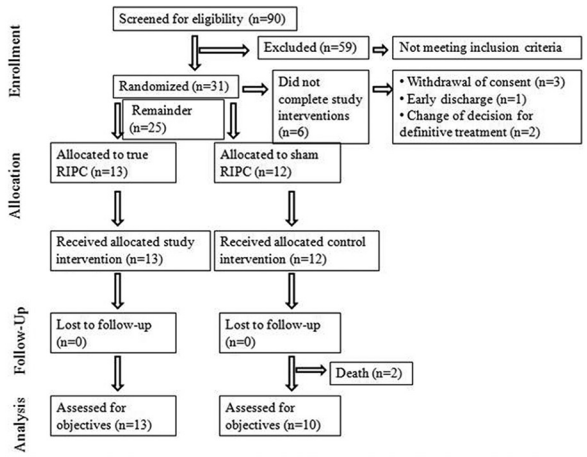


Figure 1: CONSORT flow diagram depicting the flow of patients into the study

velocity criteria of MFV >120 cm/s with LR >3, only 5 out of 25 patients were diagnosed with vasospasm using TCD, of which 1/13 (7.7%) was in the true RIPC group and 4/12 (33.3%) were in the sham RIPC group. [Table 2] All these five patients also demonstrated vasospasm on cerebral angiography.

None of the patients had vasospasm on diagnostic angiography done at presentation to the hospital. Only 13/25 patients underwent a repeat angiography, based on clinical suspicion of vasospasm [headache ($n = 7$), new-onset focal deficits ($n = 4$), and deterioration in GCS of ≥ 2 points ($n = 2$)], not attributable to other causes like hydrocephalus, re-bleed, hemodynamic instability or electrolyte abnormality. Cerebral angiography demonstrated angiographic vasospasm in 9/13 patients – 1/4 (25%) in true RIPC group and 8/9 (89%) in sham RIPC group ($P = 0.05$) [Table 2]. In the true RIPC group, one patient demonstrated mild vasospasm. Among nine patients in the sham RIPC group, three patients had mild, three had moderate, and two had severe angiographic vasospasm.

Serum biomarkers of cerebral ischemia

Serum S100B and NSE levels were estimated in 69 samples of 23 patients by enzyme-linked immunosorbent assay using commercial kits with the manufacturer's calibrators and controls. Changes in biomarker levels of both S100B and NSE over time within and between the two groups are depicted in Figure 2a and b, respectively.

There was no difference in the levels of S100B between the groups ($P = 0.56$). The trend over time for S100B was not statistically significant in both the groups ($P = 0.32$ and 0.49 in true and sham RIPC groups, respectively).

There was no difference in the levels of NSE between the groups ($P = 0.31$). Over time, the trend of NSE levels was

Table 1: Demographic characteristics of the two groups

Parameters	True RIPC (*n*=13)	Sham RIPC (*n*=12)	*P*
Age (years)	50 (41-55.50)	50 (46-54.25)	0.98
Female gender (%)	7 (53.8)	7 (58.3)	0.82
Weight (kg)	62 (54.25-65.75)	58.5 (52-69)	0.93
Height (cm)	160 (155-169)	161.5 (155-168.75)	0.65
Co-morbid illness (%)	7 (58.3)	5 (38.5)	0.32
Presence of >1 aneurysm (%)	2 (15.4)	3 (25)	0.55
GCS score at recruitment	15 (15-15)	15 (15-15)	0.73
Ictus to recruitment duration (days)	2 (2-3)	2 (1.25-3)	0.57
WFNS score at recruitment	1 (1-1)	1 (1-1)	0.50
Modified Fischer grade	3 (1.5-3)	3 (3-3.75)	0.32
Duration of temporary clip application (s)	144 (101-330.5)	212 (96-327)	0.57

Nominal variables are represented as frequencies (%) and quantitative variables as median (interquartile range). RIPC: Remote ischemic preconditioning, GCS: Glasgow Coma Scale, WFNS: World Federation of Neurological Surgeons

Table 2: Incidence of cerebral vasospasm on angiography and transcranial Doppler

Group	Angiographic vasospasm (*n*=13)		*P*	TCD vasospasm* (*n*=25)		*P*
	Absent	Present		Absent	Present	
True RIPC	3 (75)	1 (25)	0.05	12 (92.3)	1 (7.7)	0.16
Sham RIPC	1 (11.1)	8 (88.9)		8 (66.7)	4 (33.3)	

Variables are represented as frequencies (%). *On any day across the measurement period on either side. RIPC: Remote ischemic preconditioning, TCD: Transcranial Doppler

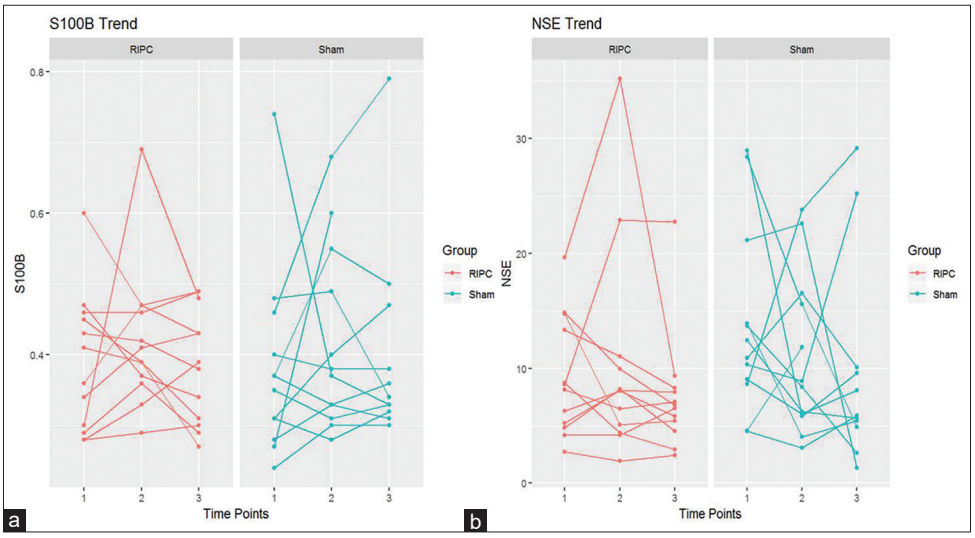


Figure 2: Trend of serum biomarkers S100B (a) and Neuron Specific Enolase (b) levels in individual patients in both the groups

not statistically significant in both the groups (P = 0.66 and 0.17 in true and sham RIPC groups, respectively).

Feasibility and safety of remote ischemic preconditioning

RIPC was feasible in all recruited patients, with a predefined minimum number of study interventions (3 sessions of true/sham RIPC). Every ischemia-reperfusion cycle was successfully completed. None of the 25 patients reported any discomfort during the true/sham RIPC maneuvers. No signs of bruising/limb ischemia/venous thrombosis/neurovascular injury were noted on any day during the study period. There were no adverse effects of RIPC on systemic hemodynamics.

Neurological outcome

Two patients (both in the sham group) died in hospital on day 5 and day 8 of ictus. There were no in-hospital nosocomial infections or other complications in either of the groups. The duration of hospital stay was longer in the sham RIPC group; median and interquartile range 7.5 (6–15.5) days versus 6 (5–6.5) days in the true RIPC group (P = 0.018). The GCS score at discharge did not differ between the groups; 15 (15–15) versus 15 (14.75–15); P = 0.446.

The GOSE score at discharge was significantly lower in the sham RIPC group; P = 0.003. The GOSE scores of patients in the true RIPC group improved over successive

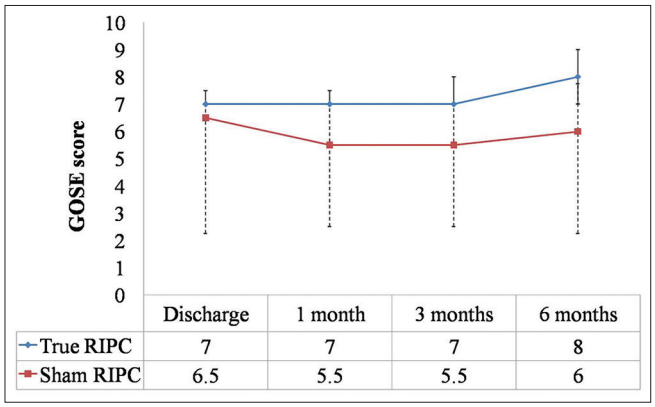
time points; $P = 0.009$. However, the GOSE scores did not change significantly in the sham RIPC group; $P = 0.847$. The GOSE scores at 1, 3, and 6 months after discharge were significantly better in the true RIPC group than the sham RIPC group; $P = 0.003$ [Figure 3].

Discussion

In this pilot RCT, we studied the cerebroprotective effects of RIPC in the context of aSAH. We evaluated vasospasm on TCD study and cerebral angiography in patients with aSAH and assessed serum biomarkers of cerebral ischemia – S100B and NSE. We also evaluated the safety of RIPC and clinical outcomes.

The overall incidence of TCD and angiographic vasospasm was 7.6% in the true RIPC group (1/13 patients) and 66.6% (8/12 patients) in the sham RIPC group, which is clinically significant. The TCD examinations revealed that 5 out of 25 patients fulfilled the predetermined criteria for vasospasm of mean CBFV >120 cm/s in the MCA and LR >3.[11] This criteria is 67% sensitive and 99% specific according to a systematic review of 26 studies that compared >25% angiographic vasospasm with mean CBFV >120 cm/s on TCD.[15]

Previous clinical studies evaluating the role of RIPC in various neurological pathologies are few and nonrandomized with small samples. Endogenous ischemic preconditioning (IPC) from the preexisting cerebrovascular steno-occlusive disease has been observed to confer protection from radiographic vasospasm after a subsequent aSAH.[16] These innate IPC pathways can be induced by various neuroprotective techniques to improve cerebral ischemic tolerance.[17,18] Direct and indirect IPC as transient sessions of sub-threshold ischemia have been shown to protect the brain from subsequent critical injury during neurosurgical interventions.[19,20] There is some preliminary evidence on the beneficial role of IPC in patients at high risk for ischemia complications such as cerebral aneurysm surgery,[21] carotid endarterectomies,[22] and carotid stenting.[23,24] Prehospital RIPC in patients with suspected stroke has been shown to ameliorate subsequent cerebral infarction.[25] Similarly, RIPC improved cerebral perfusion and prevented recurrent stroke in patients with intracranial arterial stenosis.[26] However, a systematic review of seven RCTs involving 735 participants highlighted the lack of high-quality evidence to support the routine use of RIPC in the treatment of ischemic stroke and prevention of recurrent cerebrovascular events in these patients.[27] In contrast, a recent pilot RCT investigating the role of RIPC in acute ischemic stroke patients after intravenous recombinant tissue plasminogen activator thrombolysis observed decreased NIHSS score at day 30 in the RIPC group but no difference in scores at day 90 as compared to the control group who did not receive RIPC.[28]



	Discharge	1 month	3 months	6 months
True RIPC	7	7	7	8
Sham RIPC	6.5	5.5	5.5	6

Figure 3: Representation of within and between-group changes in Glasgow Outcome Scale Extended scores (medians as lines and interquartile range as error bars)

Our study observed significant improvement in GOSE scores with RIPC overtime till 6 months after aSAH. Our findings are consistent with the findings of a previous study involving 21 patients with aSAH, which observed a similar translation of RIPC into good functional outcomes. In that study, four sessions of 5-min cycles of lower limb RIPC were associated with improved modified Rankin scale at discharge and reduced incidence of stroke and death compared to matched controls.[10]

In our study, only two patients who died had received sham RIPC. The first patient developed an ACA territory infarction following the parent vessel's inadvertent inclusion during the ACOM aneurysm clipping. In contrast, the second patient developed cerebral infarctions from bilateral severe ICA vasospasm, which was refractory to intra-arterial nimodipine treatment. Our results are in agreement with the findings of an earlier study which reported a reduction in mortality with RIPC.[10]

Cerebral vasodilatation and preservation of the cell membrane were noted following RIPC in four patients with aSAH.[8] A recent study in 50 healthy volunteers demonstrated improvement in dynamic cerebral autoregulation and alteration in serum neuroprotective factors and inflammation-related biomarkers after RIPC.[29] The cerebroprotective effect of RIPC, therefore, appears to be multifactorial from cerebral vasodilatation, cell membrane preservation, amelioration of glutamate-mediated excitotoxicity, preservation of adenosine triphosphate levels, the elevation of hypoxia-inducible transcription factor-1α,[30] and down-regulation of cerebral inflammatory cytokines.[31]

Previous trials evaluating S100B and NSE's predictive potential in aSAH for cerebral ischemia demonstrated

positive[32,33] and negative[34,35] results. These studies have not defined a threshold for serum S100B and NSE levels that prognosticates outcome. However, an increasing trend was associated with less favorable outcomes in various studies involving a diverse population of patients with cervical spine injury, traumatic brain injury, and SAH. Attenuation of these biomarkers with RIPC was observed to contribute to improved outcome.[8,36,37] One study noted serum levels of S100B >1 μg/L and NSE >30 ng/ml to be associated with unfavorable clinical outcome following aSAH.[35] In our study, S100B and NSE remained within the normal range at all time points studied, irrespective of the occurrence of vasospasm and neurological outcome. The values changed in different directions over the three time points. Surprisingly, the three patients with high values of S100B and NSE had good outcomes (GOSE at 6 months were 8 in two and 7 in one patient), and all the three patients belonged to the true RIPC group, making these biomarkers less reliable as predictors of cerebral ischemia and neurological outcome in aSAH. Our results are consistent with the findings of Moritz *et al*.,[34] that neither serum S100B nor NSE levels correlate with vasospasm or ischemia following aSAH.

In this study, apart from exploring the neuroprotective actions of RIPC in aSAH, we also studied and established the feasibility and safety of RIPC in this population, facilitating safe conductance of future larger trials to establish its effectiveness in these patients. The safety profile of RIPC was similar to that documented in patients with intracranial atherosclerosis.[24,38]

This study is the first RCT that evaluated the role of RIPC with regard to clinically important patient outcomes-cerebral vasospasm and functional outcomes along with biochemical markers of cerebral ischemia. Vasospasm, our primary outcome, was assessed clinically on TCD and cerebral angiography. Similarly, cerebral ischemia biomarkers were assessed at three-time points and GOSE at multiple time points at 1, 3, and 6 months after aSAH. However, there are certain significant limitations. Although TCD study was performed in all recruited patients, cerebral angiography was performed only in patients with clinical suspicion of vasospasm (13/25 patients). Performing angiography in all patients would have revealed the true incidence of angiographic vasospasm in our study population. Second, TCD assessment of vasospasm has inherent limitations such as availability of good transcranial window and operator dependence. These limitations were minimized by excluding patients with poor windows and TCD studies being performed by a single investigator. This study was conducted only on patients with good clinical grades of Modified Fischer and WFNS score to ensure homogeneity of the study population, and poor clinical grades, known to be at higher risk of vasospasm and DCI, were excluded. These criteria limit the generalizability of our study results. Finally, this pilot study's small sample size limits its applicability until larger trials can replicate our findings.

Conclusion

Based on our pilot study, RIPC is a feasible and safe intervention in patients with aSAH. The incidence of cerebral vasospasm was lower in those who received RIPC. RIPC did not significantly alter the serum biomarkers of cerebral ischemia. The long-term functional outcome was better in patients who received RIPC. Considering the simple nature of RIPC intervention and resultant gain in improved functional outcomes after aSAH, larger trials are needed to test and validate our preliminary findings.

Acknowledgments

Research grant: We thank the Indian Council of Medical Research (ICMR) and the Indian Society of Neuroanesthesia and Neurocritical Care (ISNACC) for supporting this study.

Participating investigators: We acknowledge the contribution of Mr. Irshadul Ameen and Miss Anusha S, Department of Neuroanesthesia and Neurocritical Care, NIMHANS, Bangalore, India, in performing the preconditioning interventions (true and sham RIPC) which make the core of this study.

We acknowledge Mr. Harish Ashwath, Department of Neurochemistry, NIMHANS, Bengaluru, for taking utmost care in ensuring the collection, processing, storage, and analysis of our study participant's blood samples.

Financial support and sponsorship

This study was funded by research grant from the Indian Council of Medical Research (ICMR) [grant No. 3/2/Jan. 2017/PG-Thesis-HRD (12)]; and the Indian Society of Neuroanesthesia and Neurocritical Care (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. 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.
2. Sharma BS, Sawarkar DP. Vasospasm: The enigma of subarachnoid hemorrhage. Neurol India 2015;63:483-5.
3. Stegmayr B, Eriksson M, Asplund K. Declining mortality from

subarachnoid hemorrhage: Changes in incidence and case fatality from 1985 through 2000. Stroke 2004;35:2059-63.
4. Findlay JM, Nisar J, Darsaut T. Cerebral vasospasm: A review. Can J Neurol Sci 2016;43:15-32.
5. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. Circulation 1993;87:893-9.
6. Lau JK, Pennings GJ, Yong A, Kritharides L. Cardiac remote ischaemic preconditioning: Mechanistic and clinical considerations. Heart Lung Circ 2017;26:545-53.
7. Gonzalez NR, Connolly M, Dusick JR, Bhakta H, Vespa P. Phase I clinical trial for the feasibility and safety of remote ischemia conditioning for aneurysmal subarachnoid hemorrhage. Neurosurgery 2014;75:590-8.
8. 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.
9. Nikkola E, Laiwalla A, Ko A, Alvarez M, Connolly M, Ooi YC, *et al.* Remote ischemic conditioning alters methylation and expression of cell cycle genes in aneurysmal subarachnoid hemorrhage. Stroke 2015;46:2445-51.
10. 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.
11. 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.
12. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. Pharm Stat 2005;4:287-91.
13. Noguchi K, Gel Y, Brunner E, Konietschke F. nparLD: An R Software Package for the Nonparametric Analysis of Longitudinal Data in Factorial Experiments. J Stat Softw 2012;50:1-23.
14. 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/. [Last accessed on 2019 Dec 23].
15. Lysakowski C, Walder B, Costanza MC, Tramèr MR. Transcranial Doppler versus angiography in patients with vasospasm due to a ruptured cerebral aneurysm: A systematic review. Stroke 2001;32:2292-8.
16. 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.
17. Klein KU, Engelhard K. Perioperative neuroprotection. Best Pract Res Clin Anaesthesiol 2010;24:535-49.
18. Ginsberg MD. Neuroprotection for ischemic stroke: Past, present and future. Neuropharmacology 2008;55:363-89.
19. Steiger HJ, Hänggi D. Ischemic preconditioning of the brain, mechanisms and applications. Acta Neurochir (Wien) 2007;149:1-10.
20. Dirnagl U, Becker K, Meisel A. Preconditioning and tolerance against cerebral ischaemia: From experimental strategies to clinical use. Lancet Neurol 2009;8:398-412.
21. Chan MT, Boet R, Ng SC, Poon WS, Gin T. Effect of ischemia preconditioning on brain tissue gases and pH during temporary cerebral artery occlusion. Acta Neurochir Suppl 2005;95:93-6.
22. Walsh SR, Nouraei SA, Tang TY, Sadat U, Carpenter RH, Gaunt ME. 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.
23. Faries PL, DeRubertis B, Trocciola S, Karwowski J, Kent KC, Chaer RA. Ischemic preconditioning during the use of the PercuSurge occlusion balloon for carotid angioplasty and stenting. Vascular 2008;16:1-9.
24. Zhao W, Meng R, Ma C, Hou B, Jiao L, Zhu F, *et al.* Safety and efficacy of remote ischemic preconditioning in patients with severe carotid artery stenosis before carotid artery stenting: A proof-of-concept, randomized controlled trial. Circulation 2017;135:1325-35.
25. Hougaard KD, Hjort N, Zeidler D, Sørensen L, Nørgaard A, Thomsen RB, *et al.* Remote ischemia preconditioning as an adjunct therapy to thrombolysis in patients with acute ischemia stroke: A randomized trial. Stroke 2014;45:159-67.
26. 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.
27. Zhao W, Zhang J, Sadowsky MG, Meng R, Ding Y, Ji X. Remote ischaemic conditioning for preventing and treating ischaemic stroke. Cochrane Database Syst Rev 2018;7:CD012503.
28. Che R, Zhao W, Ma Q, Jiang F, Wu L, Yu Z, *et al.* rt-PA with remote ischemic postconditioning for acute ischemic stroke. Ann Clin Transl Neurol 2019;6:364-72.
29. Guo ZN, Guo WT, Liu J, Chang J, Ma H, Zhang P, *et al.* Changes in cerebral autoregulation and blood biomarkers after remote ischemia preconditioning. Neurology 2019;93:e8-19.
30. Li Y, Ren C, Li H, Jiang F, Wang L, Xia C, *et al.* Role of exosomes induced by remote ischemic preconditioning in neuroprotection against cerebral ischemia. Neuroreport 2019;30:834-41.
31. Koch S, Gonzalez N. Preconditioning the human brain: Proving the principle in subarachnoid hemorrhage. Stroke 2013;44:1748-53.
32. Sanchez-Peña P, Pereira AR, Sourour NA, Biondi A, Lejean L, Colonne C, *et al.* S100B as an additional prognostic marker in subarachnoid aneurysmal hemorrhage. Crit Care Med 2008;36:2267-73.
33. Mabe H, Suzuki S, Mase M, Umemura A, Nagai H. Serum neuron-specific enolase levels after subarachnoid hemorrhage. Surg Neurol 1991;36:170-4.
34. Moritz S, Warnat J, Bele S, Graf BM, Woertgen C. The prognostic value of NSE and S100B from serum and cerebrospinal fluid in patients with spontaneous subarachnoid hemorrhage. J Neurosurg Anesthesiol 2010;22:21-31.
35. Oertel M, Schumacher U, McArthur DL, Kästner S, Böker DK. S-100B and NSE: Markers of initial impact of subarachnoid hemorrhage and their relation to vasospasm and outcome. J Clin Neuro Sci 2006;13:834-40.
36. 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.
37. 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.
38. Li S, Ma C, Shao G, Esmail F, Hua Y, Jia L, *et al.* Safety and feasibility of remote limb ischemia preconditioning in patients with unilateral middle cerebral artery stenosis and healthy volunteers. Cell Transplant 2015;24:1901-11.