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Authors

Gonzalez, Nestor R
Connolly, Mark
Dusick, Joshua R
et al.

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Phase I Clinical Trial for the Feasibility and Safety of Remote Ischemic Conditioning for Aneurysmal Subarachnoid Hemorrhage

Nestor R. Gonzalez, MD
Mark Connolly, BS
Joshua R. Dusick, MD
Harshal Bhakta
Paul Vespa, MD

Department of Neurosurgery, David Geffen School of Medicine at the University of California, Los Angeles, California

Correspondence:
Nestor R. Gonzalez, MD,
Associate Professor of Neurosurgery and Radiology,
David Geffen School of Medicine at UCLA,
10833 LeConte Ave, Room 18-251 Semel, Los Angeles, CA 90095-7039.
E-mail: ngonzalez@mednet.ucla.edu

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BACKGROUND: Remote ischemic conditioning (RIC) is a powerful endogenous mechanism whereby a sublethal ischemic stimulus confers a protective benefit against a subsequent severe ischemic insult. RIC has significant potential clinical implications for the prevention of delayed ischemic neurological deficit after aneurysmal subarachnoid hemorrhage (aSAH). Although RIC has been extensively investigated in animal models, it has not been fully evaluated in humans.

OBJECTIVE: To assess the feasibility and safety of RIC for aSAH in a phase I clinical trial.

METHODS: Consecutive patients hospitalized for treatment of an aSAH who met the inclusion/exclusion criteria were approached for consent. Enrolled patients received up to 4 RIC sessions on nonconsecutive days. Primary end points were the development of a symptomatic deep venous thrombosis, bruising, or injury to the limb and request to stop by the patient or surrogate. The secondary end points were the development of new neurological deficits or cerebral infarct, demonstrated by brain imaging after enrollment, and neurological deficit and condition at follow-up.

RESULTS: Twenty patients were enrolled and underwent 76 RIC sessions, 75 of which were completed successfully. One session was discontinued when the patient became confused. No patient developed a deep venous thrombosis or injury to the preconditioned limb. No patient developed delayed ischemic neurological deficit during enrollment. At follow-up, median modified Rankin Scale score was 1 and Glasgow Outcome Scale score was 5.

CONCLUSION: The RIC procedure was well tolerated and did not cause any injury. RIC for aSAH warrants investigation in a subsequent pivotal clinical trial.

KEY WORDS: Clinical trial, Remote ischemic conditioning, Subarachnoid hemorrhage

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Ischemic preconditioning is a powerful endogenous mechanism whereby a mild ischemic stress to a tissue renders it resistant to subsequent severe ischemia. This protective mechanism has also been demonstrated when a low-risk organ such as a limb is used to induce the ischemic transient stress yet still confers a protective benefit to more critical organs such as the heart or brain, a phenomenon known as remote ischemic conditioning (RIC).[1-6] RIC has potential clinical applications in the prevention of cerebral ischemia because it can be applied without exposing the brain to unnecessary risk. The mechanism of ischemic protection underlying RIC is likely multifactorial and includes a downregulation of genes associated with inflammation,[7,8] a reduction in glutamatergic excitotoxicity,[9,10] blockage of apoptotic neuronal pathways,[11,12] and an increase in blood flow to the ischemic penumbra.[13]

So far, the majority of clinical applications of ischemic preconditioning have focused on the prevention of myocardial infarction, showing potential protective benefit before coronary bypass and percutaneous angioplasty and stenting procedures.[1,14,15] There have been a limited number of attempts to apply RIC for cerebral protection, the results of which have been inconsistent.[5,16]

ABBREVIATIONS: aSAH, aneurysmal subarachnoid hemorrhage; **DIND,** delayed ischemic neurological deficit; **RIC,** remote ischemic conditioning; **TCD,** transcranial Doppler

To successfully translate the powerful effects of RIC observed in animal studies into clinical practice, several challenges need to be overcome in a systematic manner: (1) A remote stimulus that can generate transient but effective ischemia must be identified and demonstrated to induce ischemia; (2) it must be applied to a condition in which the time window of likely ischemia allows the use of preconditioning; (3) the physiological effects associated with the RIC maneuvers should be evaluated; (4) the technique selected for induction of remote preconditioning should be tested for safety and tolerability; and (5) the effects of remote ischemic preconditioning should be compared with standard management of that condition in a randomized trial. We have performed preliminary studies addressing the first 3 steps of this translational strategy. Our prior work has demonstrated that lower-limb transient ischemia is feasible and produces local metabolic changes consistent with sublethal ischemia.[17] The rationale for selecting patients with aneurysmal subarachnoid hemorrhage (aSAH) to prevent delayed ischemic neurological deficit (DIND) is based on the well-defined time course of vasospasm and DIND after aneurysmal rupture and has been discussed extensively.[18] In a pilot study, we demonstrated the cerebral vascular and metabolic effects of the RIC maneuvers with lower-limb ischemia.[19] Finally, to test the hypothesis that RIC by induction of transient lower-limb ischemia is a feasible and safe strategy in patients hospitalized for the treatment of aSAH, we present here the results of a prospective phase I clinical trial to test the safety and feasibility of RIC induced by transient lower-limb ischemia in patients with aSAH.

TABLE 1. Inclusion and Exclusion Criteria for Enrollment in the Trial

Inclusion criteria
Patient age, 18 to 80 y
Patient capable of providing consent or accompanied by appropriate surrogate for consent
Subarachnoid hemorrhage confirmed on computed tomography or Lumbar puncture with aneurysm origin confirmed by computed tomography angiography or angiography
Aneurysm has been protected by clipping or coiling
Time of enrollment within 72 h of subarachnoid hemorrhage
Informed consent signed by patients or surrogate
Exclusion criteria
Unprotected intracranial aneurysm or aneurysms
History of peripheral vascular disease, lower-extremity bypass, or physical examination findings of vascular disease
History of deep vein thrombosis or physical examination findings of deep vein thrombosis
Pregnancy
Parenchymal or intracerebral hemorrhage
History of peripheral neuropathy or physical examination findings of peripheral neuropathy

METHODS

Study Design and Patients

This was a single-center, phase I study to assess the feasibility and safety of RIC as a prophylactic treatment for DIND. Patients with aSAH confirmed by computed tomography, magnetic resonance, or catheter angiography and single or multiple aneurysms protected by endovascular coiling or surgical clipping who met the inclusion and exclusion criteria (Table 1) were considered for enrollment. Although the intention was to enroll only patients within 72 hours of hemorrhage, many patients are admitted after this window. For this reason, the decision was made to extend the enrollment widow to 14 days after hemorrhage. Consecutive eligible patients or their legal representatives were approached for consent. This study and the materials used for enrollment and consent were approved by the university institutional review board.

The enrollment goal for this trial was guided by the confidence interval approach described by Thabane et al.[20] A safety concern for RIC is the development of a deep venous thrombosis (DVT) from the repeated lower-limb compression. However, DVTs can develop as a side effect of routine ICU care and therefore confound the assessment of safety. The sample size calculation was based on a 95% confidence interval for the proportion of patients who develop a DVT with an upper bound of 0.25. The estimated incidence of DVT for unscreened patients with aSAH was based on a reported value of 12%,[21] giving a required sample size of 15 patients.

Preconditioning Protocol

Patients underwent RIC sessions on nonconsecutive days until any 1 of the following 3 conditions were met: 4 sessions had been completed, the patient reached a clinical end point (development of a DVT or request to stop), or the patient was discharged. The leg that had not received any catheter treatments (catheter angiogram or venous cooling catheter devices) was designated for preconditioning. In preparation for each session, the dorsalis pedis artery was identified with the aid of a pulsed Doppler and marked with a permanent marker. A large blood pressure cuff was placed around the leg and inflated to 20 mm Hg above the patient's systolic blood pressure, and the connecting tubes were clamped with locking forceps. The pedal pulsed Doppler was then used to verify that the signal had been obliterated, confirming cessation of blood flow. If the pulse was detected, the blood pressure cuff was inflated further within the comfort level of the patient. After successful induction of ischemia, the cuff pressure was maintained for 5 minutes while the absence of a pedal pulse was periodically verified. After 5 minutes of ischemia, the blood pressure cuff was deflated, and the limb was allowed to reperfuse. Reperfusion was confirmed with the pedal pulsed Doppler. After 5 minutes, the cycle of ischemia/reperfusion was repeated an additional 3 times. After the completion of 4 cycles of ischemia and reperfusion, the cuff was removed and the leg was inspected for bruising or other injury. A complete preconditioning session is defined as 4 rounds of verified ischemia and perfusion.

Primary and Secondary End Points

The primary end points to assess the feasibility and safety of the RIC for aSAH were (1) the development of a symptomatic DVT in the limb used for preconditioning that is temporally related to the procedure or bruising/damage to the limb during or after the preconditioning treatment and (2) request by the patient or surrogate to cease the remote ischemic preconditioning treatment.

TABLE 2. Vital Signs and Additional Monitoring Measurements

	Units
Vital sign	
Heart rate	bpm
Systolic blood pressure	mm Hg
Diastolic blood pressure	mm Hg
Mean arterial pressure	mm Hg
Respiratory rate	breaths/min
Temperature	°C
Additional monitoring	
Central venous pressure	mm Hg
Pain	0-10, Ordinal
Intracranial pressure	mm Hg
Right middle cerebral artery mean cerebral blood flow velocity	cm/s
Left middle cerebral artery mean cerebral blood flow velocity	cm/s
Right middle cerebral artery pulsatility index	. . .
Left middle cerebral artery pulsatility index	. . .

The secondary end points were the development of new neurological deficits or cerebral infarct demonstrated by brain imaging after enrollment and neurological deficit and condition at follow-up (modified Rankin Scale and Glasgow Outcome Scale scores).

Physiological Data Collection

Vital signs and additional monitoring data (Table 2) were collected at baseline and at each subsequent inflation and deflation, for a total of 9 measurements for each variable per RIC session. In addition to routine monitoring, patients received continuous bilateral transcranial Doppler (TCD) monitoring during the entirety of the preconditioning session when possible. If the patient had an external ventricular drain placed, it was closed before the start of the session and opened if the intracranial pressure exceeded the designated clinical goal.

Systolic and diastolic blood pressures, mean arterial pressure, central venous pressure, intracranial pressure, heart rate, and respiratory rate were collected from the bedside monitors. Cerebral blood flow velocity and pulsatility indexes of both middle cerebral arteries were measured with TCD. Measurements were taken from the readings of the bedside monitors and TCD machine at the midpoint of each period of ischemia (2.5 minutes after cuff inflation) and reperfusion (2.5 minutes after cuff deflation). Data were recorded manually.

TABLE 3. Patient Demographics and Past Medical History

	Value
Mean age, y	53
Ethnicity, n	
Hispanic	7
Non-Hispanic	13
Race, n	
White	13
Black	3
Asian	4
Pacific Islander	0
Native American	0
Medical history, n	
Diabetes mellitus	3
Hypertension	12
Hyperlipidemia	2
Past social history, n	
Smoking	7
Alcohol	6
Unknown	1

TABLE 4. Admission Status

Assessment	n
Hunt and Hess	
1	2
2	4
3	5
4	4
5	5
Fisher	
1	0
2	2
3	5
4	13

Statistical Methods

All rational vital signs and additional monitoring data were analyzed with a repeated-measures analysis of variance. The 3 repeated-measures analysis of variance treatments considered were inflation, time point (baseline, first inflation, first deflation, etc), and session (first session, second session, etc). Because the pain scale was ordinal, changes were

TABLE 5. Protocol Adherence

	n
Days to enrollment after hemorrhage	
1	0
2	2
3	6
4	2
5	2
6	3
7	3
>7	2
No. of sessions completed	
1	0
2	1
3	3
4	16
No. of sessions not completed	
1	1

TABLE 6. Primary End Points

End Point	n	Notes
Deep vein thrombosis	2	11 and 28 d after final session
Bruising or damage to limb	0	
Request to stop preconditioning	0	

assessed by use a Wilcoxon signed-rank test to compare the median pain scale for each patient between inflation and deflation. To account for the repeated comparisons, the Holm-Bonferroni correction was applied to an α of 0.05 for all statistical tests. All statistical calculations were performed with IBM SPSS Statistics, 64-bit edition, version 21, release 21.0.0.0.

RESULTS

Patient Demographics and Admission Status

Twenty patients were enrolled and received RIC treatment. Of these patients, 6 (30%) were male and 14 (70%) were female. The age range was 22 to 77 years of age (mean, 53 years; SD, 9.27 years). Complete patient demographics are detailed in Table 3.

Enrolled patients had a median Hunt and Hess score of 3 and a median Fisher score of 4 at admission (Table 4).

Adherence to the Protocol

A total of 8 patients (40%) were enrolled within 72 hours after hemorrhage, and 18 (90%) patients were enrolled within 7 days after hemorrhage. Two patients (10%) were not enrolled within 7 days (9 and 14 days). Protocol adherence is summarized in Table 5.

TABLE 7. Secondary End Points

End Point	
Stroke, n	
Before enrollment	2
During remote ischemic preconditioning + 48 h	0
>48 h after remote ischemic preconditioning completion	3
Modified Rankin Scale score, n	
0 = No symptoms	7
1 = No significant disability	6
2 = Slight disability	
3 = Moderate disability	
4 = Moderately severe disability	2
5 = Severe disability	4
6 = Dead	1
Glasgow Outcome Scale score, n	
1 = Death	1
2 = Persistent vegetative state	1
3 = Severe disability	5
4 = Moderate disability	
5 = Low disability	13
Follow up time, mo	
Mean	5.7
SD	6.9

Primary End Points

During the course of the preconditioning treatment, no patient developed a symptomatic DVT or bruising or injury related to the RIC procedure. Two patients eventually developed symptomatic DVTs 11 and 28 days after the completion of the final conditioning session. Both patients had catheters placed in the ipsilateral femoral vein for treatment of hyperthermia after the conclusion of the RIC treatments and before the development of the DVTs.

In general, the RIC procedure was well tolerated by the patients. A total of 76 RIC sessions were attempted for the enrolled patients. Of these, 75 were completed and 1 was discontinued early as a result of an episode of delirium in the patient, which limited cooperation with the procedure. Three patients (15%) received only 3 RIC sessions: 1 patient died of complications related to the initial aSAH (Hunt and Hess grade 5, Fisher grade 4), and 2 patients were discharged before all 4 sessions could be completed (Table 6).

Secondary End Points

Of the enrolled patients, 5 developed DINDs. Two patients suffered cerebral infarcts before enrollment, and 3 others suffered cerebral infarcts beyond 48 hours after the completion of the final conditioning session. After discharge, patients were followed up as part of their routine care. The mean follow-up time was 5.7 months with a standard deviation of 6.9 months. At follow-up, patients had a median modified Rankin Scale score of 1 and a median Glasgow Outcome Scale score of 5 (Table 7).

Vital Signs and Physiological Monitoring

Analysis of the vital signs and additional monitoring data found no statistically significant change for any of the 3 repeated-measures analysis of variance treatments (inflation, time point, session). The time course of the vital signs and additional monitoring data are shown for inflation in Figure 1 and for time point in Figures 2 and 3.

DISCUSSION

The goal of this phase I clinical trial was to determine whether RIC was safe and feasible as a prophylactic treatment for DIND in patients with aSAH. Patients who met the inclusion and exclusion criteria and gave consent to participate in the study received up to 4 sessions of RIC every other day or until discharge. We found that no patient developed bruising or injury to the conditioned limb, no patient developed symptomatic DVTs that were spatially or temporally related to the preconditioning procedure, and no patient requested that the preconditioning procedure be stopped. These results suggest that the RIC is safe and feasible for patients with acute aSAH.

RIC is a powerful endogenous mechanism whereby a transient, sublethal ischemia to 1 tissue can confer protective

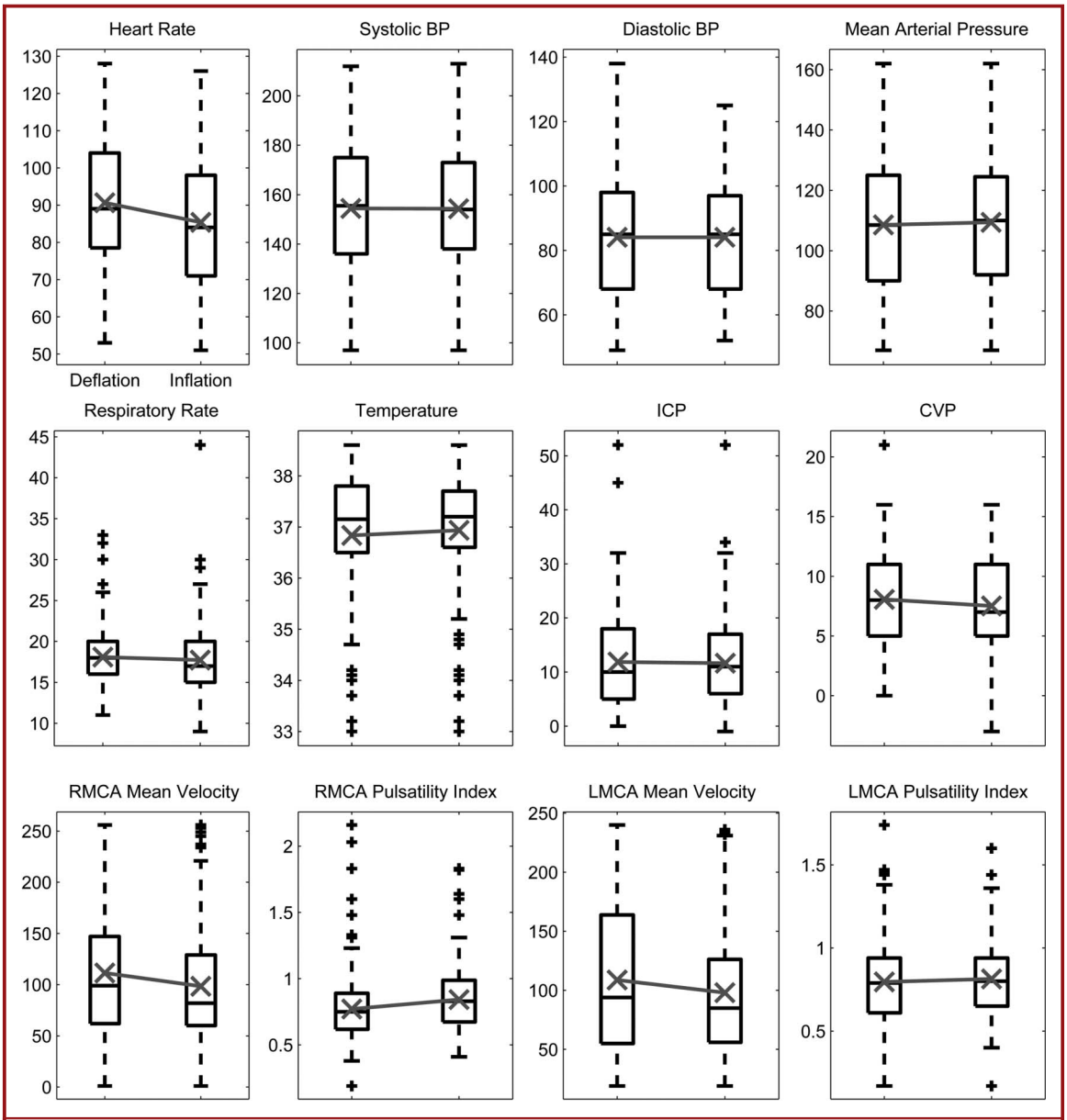

FIGURE 1. *The distribution across deflation and inflation for each vital sign and physiological signal monitored. Black box plots show the median, 25th and 75th percentiles, range, and outliers (+). Overlaid in gray is the mean value. BP, blood pressure; CVP, central venous pressure; ICP, intracranial pressure; LMCA, left middle cerebral artery; RMCA, right middle cerebral artery.*

benefits to another tissue. Through a multifactorial process, RIC induces anti-ischemia mechanisms such as the down-regulation of proinflammatory genes, the upregulation of anti-inflammatory genes,[7] and the upregulation of genes associated with cytoprotection, DNA repair, growth, and metabolism.[22] The systemic effect of these anti-ischemia mechanisms, through humoral and neural pathways, makes RIC a promising potential prophylactic treatment for acute ischemia to the brain.

To effectively investigate clinical application of RIC, it was necessary to obtain evidence that the proposed procedure was capable of creating effective, but sublethal, ischemia in the limb; to identify a time window at which a patient is at an elevated risk of stroke; and to demonstrate that the procedure could induce a detectable physiological change. To address the first point, we conducted a preliminary study using muscle microdialysis during the RIC procedure.[17] We found that after the prolonged lower-limb ischemia there was a significant increase in the lactate concentration and ratio of lactate

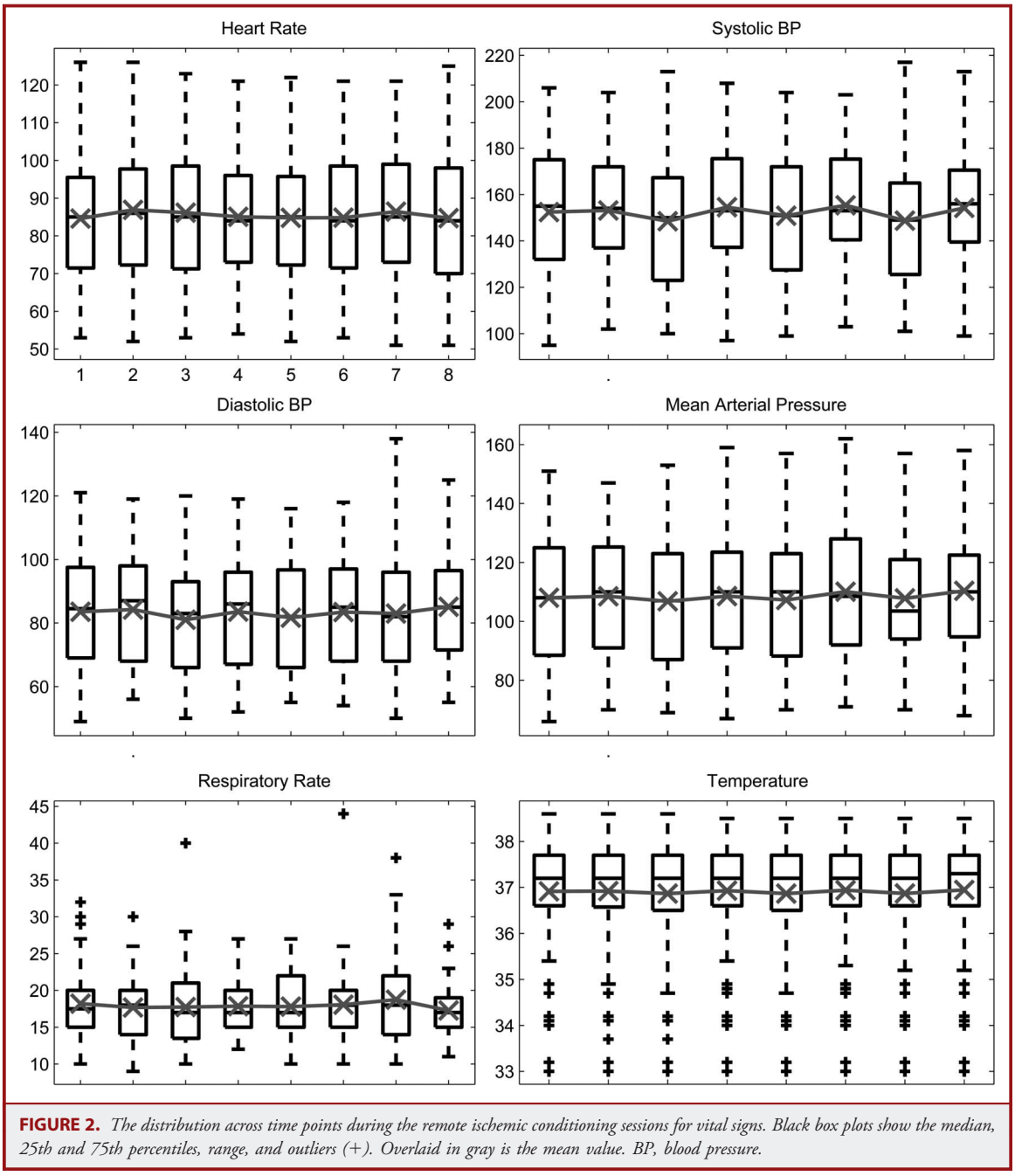

FIGURE 2. *The distribution across time points during the remote ischemic conditioning sessions for vital signs. Black box plots show the median, 25th and 75th percentiles, range, and outliers (+). Overlaid in gray is the mean value. BP, blood pressure.*

to pyruvate, both markers of ischemia. Moreover, there was no significant variation of glycerol, indicating that the ischemia did not cause any appreciable permanent cell damage.

With an RIC procedure capable of producing sublethal ischemia, it was necessary to identify a condition and time window in which the procedure has the potential to provide a benefit. After an aSAH, there is a well-defined period when patients are at an elevated risk of DIND and may benefit from RIC as a prophylactic measure.[18] Additionally, the controlled conditions and close monitoring afforded by their treatment in the neurocritical care unit were ideal to investigate the safety and feasibility of RIC for aSAH.

To assess whether the RIC procedure induced any physiological change, we collected intracranial pressure and TCD waveforms and

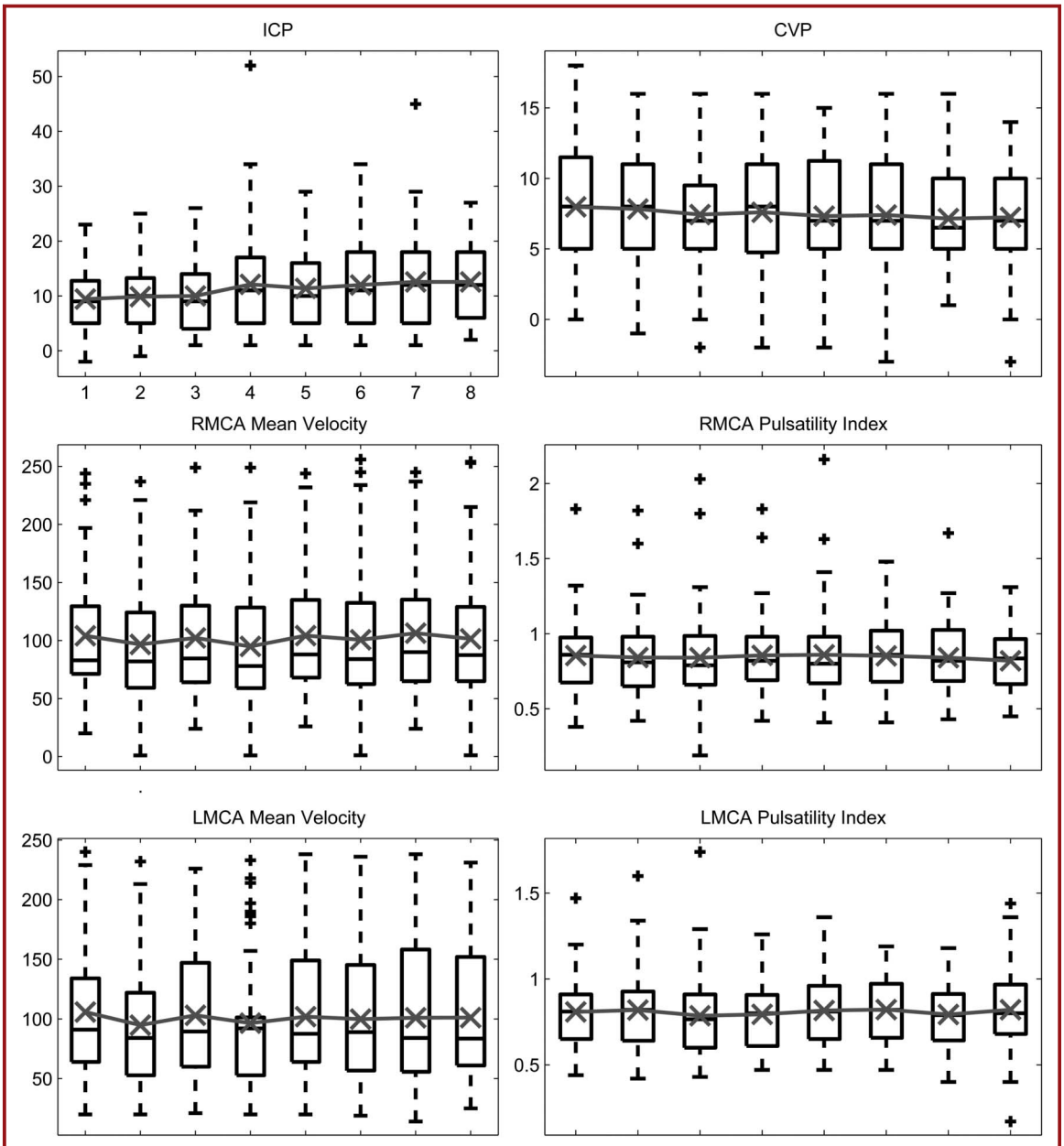

FIGURE 3. *The distribution across time points during the remote ischemic conditioning sessions for other physiological monitoring measurements (intracranial pressure [ICP], central venous pressure [CVP], bilateral cerebral blood flow velocities, and pulsatility indexes). Black box plots show the median, 25th and 75th percentiles, range, and outliers (+). Overlaid in gray is the mean value. LMCA, left middle cerebral artery; RMCA, right middle cerebral artery.*

brain microdialysis.[19] With these data, we demonstrated that during the preconditioning procedure the intracranial pressure and TCD waveform changes were consistent with those observed during hypercapnic vasodilation.

The next step was to test the hypothesis that RIC is safe and feasible in the setting of aSAH. In this study, 20 patients were enrolled within a median of 4 to 5 days (range, 1-13 days) after aSAH. Although only 8 patients were enrolled before the beginning of the window of elevated risk of DIND (day 3), 18 were enrolled before the period of maximum DIND risk (days 7-10). The 2 patients who were enrolled later than day 7 were both transferred from outside hospitals.

In the cohort reported here, no patient reached the first primary end point: bruising or damage to the limb or symptomatic DVT

formation that could be temporally related to the RIC sessions. Two patients eventually developed symptoms of DVT that were confirmed with venous Doppler. However, these events occurred >1 week after the completion of all 4 RIC sessions and after cooling catheters had been applied to the ipsilateral femoral vein as part of the patients' treatment unrelated to the study. The reported association between endovascular cooling treatments and an increased risk of deep venous[23,24] and inferior vena cava[25] thrombus formation supports that the DVTs were not related to the RIC procedure. However, this is a point to consider for the clinical application of RIC because critical and, specifically, aSAH patients have an increased incidence of DVT.[21,26]

The second end point of this study addressed the patients' tolerability of the procedure. As reported above, 75 of the attempted 76 RIC sessions were completed successfully. One session was aborted early when it was observed that the patient was experiencing confusion and became uncooperative. In the other 75 RIC sessions, no conscious patient requested that the procedure be stopped, although some did express that the procedure caused them a mild but tolerable level of discomfort. A previous clinical trial of RIC for aSAH by Koch et al[6] also found that the procedure was tolerated by the patients and that no patient developed any neurovascular injury. However, the results reported by Koch et al are limited. In their study, the preconditioning was performed by inflating a large blood pressure cuff around the limb to 200 mm Hg without confirming the cessation of blood flow to the limb (eg, by pedal Doppler). In patients with atherosclerotic vessels, those who are obese, or those with elevated blood pressure as part of their treatment, 200 mm Hg may have been insufficient to produce ischemia. By not confirming that ischemia was produced in the limb, the study by Koch et al did not fully assess the safety and tolerability of RIC.

Although the design of our study was not intended to assess the efficacy of RIC in preventing DIND after aSAH, the preliminary findings were promising. No patient developed permanent DINDs within 3 days of a successful RIC session. Three patients went on to develop cerebral infarcts 3 and 5 days after their final RIC session. These findings agree with previous reports that the effect of ischemic conditioning decreases over time and has a limited duration.[27] This result also suggests that the RIC protocol may be extended to cover the entire time window in which patients are at an increased risk of DIND. The other secondary end point was patient outcome at follow-up. At an average follow-up time of 5.7 months, patients enjoyed a median Glasgow Outcome Scale score of 5 and modified Rankin Scale score of 1. Although these findings are encouraging, the cohort was too small and heterogeneous to draw any specific conclusion.

Because many of the patients enrolled in this study were sedated, comatose, or otherwise unable to provide feedback about any discomfort created by the procedure, we also recorded the patients' vital signs and other physiological monitoring information. We found that there was no significant change in any monitoring modality in response to the RIC. Although not associated with a specific end point in this study, these findings add support to the safety and feasibility of applying RIC in unconscious patients, as was reported in a study of RIC before carotid endarterectomy by Walsh et al.[16]

Taken together, these results demonstrate that RIC is safe and feasible for patients hospitalized for treatment of aSAH.

Limitations

This study was limited by the prespecified number of RIC sessions. Although we were able to demonstrate the feasibility and safety of RIC for aSAH over a period of 7 days, this does not necessarily extrapolate to the feasibility and safety over the entire period of elevated risk of DIND. However, this design permitted the identification of the durability of a potential protective effect, which appears to be 48 hours after each session. Future studies on the clinical application of RIC for aSAH should address this by continuing the nonconsecutive RIC sessions throughout the duration of the patients' stay in the intensive care unit, along with a continued evaluation of the safety and tolerability of the procedure.

CONCLUSION

We conducted a phase I clinical trial to assess the feasibility and safety of RIC as a prophylactic treatment for DIND after aSAH. We found that the RIC procedure was well tolerated by patients and did not cause any injury. No patient developed DIND during enrollment in the trial, and patients had promising outcomes at follow-up. RIC for aSAH should be investigated in a subsequent pivotal clinical trial.

Disclosure

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COMMENTS

The authors propose an interesting study on the feasibility and safety of multisession remote ischemic conditioning (RIC) via lower-extremity blood pressure cuff inflation in subarachnoid hemorrhage patients as prophylaxis against delayed ischemic neurological deficits. The central hypothesis behind this study is an intriguing one, and the concept of ischemic conditioning is well accepted in the acute stroke arena, making this project a logical extension. The authors lay out 5 goals of their RIC clinical trial. The main goal of this article was to focus on proof of safety and tolerability of RIC. We note the theoretical possibility for deep vein thrombosis and agree that strict monitoring criteria are vital to patient safety during this study. Overall, this type of preventive treatment strikes us as simple to implement and intuitively safe. Hence, the cerebrovascular community will follow this trial through its stages with great anticipation.

Ryan P. Morton
Louis J. Kim
Seattle, Washington

In this single-institution phase I clinical trial, the authors have studied the feasibility of utilizing remote ischemic conditioning (RIC) in patients with aneurysmal subarachnoid hemorrhage. In this cohort of 20 patients, there were no untoward physiological sequelae resulting from this prophylactic treatment for cerebral vasospasm. Although 2 patients developed delayed ischemic neurological deficits after the discontinuation of RIC, the purpose of this pilot study was merely to evaluate safety and feasibility. Cerebral vasospasm remains a menacing and potentially devastating problem, and much work remains to be done to improve patient outcomes. Moving forward, it will be interesting to see whether RIC is of any benefit as a prophylactic treatment.

Adam S. Arthur
Memphis, Tennessee