Remote ischemic conditioning for acute ischemic stroke part 2: Study protocol for a randomized controlled trial

Kentaro Ishizuka1*, Takao Hoshino1, Sono Toi1, Takaumi Mizuno1, Megumi Hosoya1, Moeko Saito1, Yasuto Sato2, Yoshibi Yagita3, Kenichi Todo4, Manabu Sakaguchi5, Takashi Ohashi6, Kenji Maruyama7, Shuji Hino8, Yutaka Honma9, Ryosuke Doijiri10, Hiroshi Yamagami11, Yasuyuki Iuchi12, Teruyuki Hirano13, Kazumi Kimura14, Takanari Kitazono15 and Kazuo Kitagawa1*

1 Department of Neurology, Tokyo Women's Medical University School of Medicine, Tokyo, Japan, 2 Department of Public Health, Tokyo Women's Medical University School of Medicine, Tokyo, Japan, 3 Department of Stroke Medicine, Kawasaki Medical School, Okayama, Japan, 4 Department of Neurology, Osaka University Graduate School of Medicine, Osaka, Japan, 5 Department of Neurology, Osaka General Medical Center, Osaka, Japan, 6 Department of Neurology, Tokyo Women's Medical University Yachiyo Medical Center, Chiba, Japan, 7 Department of Neurology, Toda Chuo General Hospital, Saitama, Japan, 8 Department of Neurology, Saitama Red Cross Hospital, Saitama, Japan, 9 Department of Neurology, Showa General Hospital, Tokyo, Japan, 10 Department of Neurology, Iwate Prefectural Central Hospital, Iwate, Japan, 11 Department of Stroke Neurology, National Hospital Organization, Osaka National Hospital, Osaka, Japan, 12 Department of Neurology, The Jikei University School of Medicine, Tokyo, Japan, 13 Department of Stroke and Cerebrovascular Medicine, Kyorin University, Tokyo, Japan, 14 Department of Neurology, Nippon Medical School, Tokyo, Japan, 15 Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Background: Remote ischemic conditioning (RIC) refers to the application of repeated short periods of ischemia intended to protect remote areas against tissue damage during and after prolonged ischemia.

Aim: We aim to evaluate the efficacy of RIC, determined by the modified Rankin Scale (mRS) score at 90 days after stroke onset.

Design and methods: This study is an investigator-initiated, multicenter, prospective, randomized, open-label, parallel-group clinical trial. The sample size is 400, comprising 200 patients who will receive RIC and 200 controls. The patients will be divided into three groups according to their National Institutes of Health Stroke Scale score at enrollment: 5–9, mild; 10–14, moderate; 15–20, severe. The RIC protocol will be comprised of four cycles, each consisting of 5 min of blood pressure cuff inflation (at 200 mmHg or 50 mmHg above the systolic blood pressure) followed by 5 min of reperfusion, with the cuff placed on the thigh on the unaffected side. The control group will only undergo blood pressure measurements before and after the intervention period. This trial is registered with the UMIN Clinical Trial Registry (https://www.umin.ac.jp/; UMIN000046225).

Study outcome: The primary outcome will be a good functional outcome as determined by the mRS score at 90 days after stroke onset, with a target mRS
score of 0–1 in the mild group, 0–2 in the moderate group, and 0–3 in the severe group.

**Discussion:** This trial may help determine whether RIC should be recommended as a routine clinical strategy for patients with ischemic stroke.

**KEYWORDS**
acute ischemic stroke, remote ischemic conditioning (RIC), neurological severity, good functional outcome, randomized controlled trial

## Introduction

In recent years, hyperacute reperfusion treatment has progressed remarkably due to the establishment of recombinant tissue-type plasminogen activator (rt-PA) and endovascular treatment (EVT) (1, 2). Although Japan is proceeding with a plan to accelerate the development of an efficient care system for stroke patients (forming new stroke centers and stroke care units), only 6–8% of patients can receive hyperacute reperfusion therapy (3). In Japan, edaravone has been used as an effective method to reduce ischemic insults. However, it is not widely used internationally; it has yet to demonstrate sufficient efficacy (4).

Remote ischemic conditioning (RIC) is a therapeutic strategy in which several cycles of brief focal ischemia, followed by reperfusion in the arms or legs, confer protection against the more severe detrimental effects of ischemia in target organs (5–8). Although the underlying mechanisms are not fully understood, current evidence indicates that RIC reduces inflammation, oxidative stress, and cerebral edema, mediated by humoral, immunoregulatory, and neurotrophic factors (9). Although the clinical application of RIC in patients with acute ischemic stroke has been attempted, its efficacy has not yet been validated (10–12). Moreover, no clinical trials have been conducted on acute ischemic strokes in Japan.

One reason for the inability to confirm the efficacy of RIC in previous studies may be the use of a unified definition of a good outcome as assessed by the modified Rankin Scale (mRS), regardless of the neurological severity of the enrolled patients upon admission. Another reason may be the lack of an established RIC protocol. Therefore, this study aims to evaluate the efficacy of an RIC protocol based on recent literature, determined by the mRS score at 90 days after stroke onset, with a good outcome defined according to the severity at enrollment.

**Abbreviations:** EVT, endovascular treatment; FAS, full analysis set; MACE, major adverse cardiovascular events; MRI, magnetic resonance imaging; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; RIC, remote ischemic conditioning; RICAIS, Remote Ischemic Conditioning for Acute Ischemic Stroke; rt-PA, recombinant tissue-type plasminogen activator.

## Methods and analysis

### Design

Remote Ischemic Conditioning for Acute Ischemic Stroke (RICAIS) part 2 is an investigator-initiated, multicenter, prospective, randomized, open-label, parallel-group clinical trial targeted at patients with acute ischemic strokes. The protocol is registered with the UMIN Clinical Trial Registry (UMIN000046225).

### Patient population

We will recruit patients diagnosed as having had an acute ischemic stroke via brain MRI and/or CT. All 14 Japanese stroke centers and their prehospital regional centers will be invited to participate. The inclusion and exclusion criteria are listed in **Table 1**, and the baseline assessments and study procedures are provided in **Table 2** and **Figure 1**. Participants need to show a defined ischemic lesion on magnetic resonance imaging (MRI). Patients who received alteplase treatment or mechanical thrombectomy can be enrolled 12 h after the intervention. All patients will provide written informed consent in the acute phase. If the research participant is an adult who is objectively judged to lack the capacity to give informed consent due to a speech or writing impairment caused by a stroke, the consent may be obtained from the legal representative.

### Randomization

The patients will be divided according to their National Institutes of Health Stroke Scale (NIHSS) score at enrollment: 5–9, mild; 10–14, moderate; 15–20, severe. Randomization will be performed using a predefined table generated by a computer program, and the results of the assignment will be immediately sent to the physician in charge via a computer. To eliminate bias, the assigned study groups will not be disclosed to the evaluating physician during evaluation of mRS at 90 days post stroke. All
TABLE 1  Inclusion and exclusion criteria.

| Inclusion criteria                                                                 | Exclusion criteria       |
|------------------------------------------------------------------------------------|--------------------------|
| • Patients hospitalized in participating institutions                              | • mRS >2 before stroke onset |
| • Male and female patients (age range, 20–90 years)                                 | • Planned intravenous rt-PA and/or EVT after registration |
| • Diagnosed as acute ischemic stroke by performing brain MRI and/or CT              | • Within 12 h after rt-PA administration or EVT |
| • Within 48 h after stroke onset                                                  | • Systolic blood pressure ≥180 mmHg |
| • NIHSS scores range from 5 to 20 at registration                                  | • History of PAD          |
| • Tolerance to systemic blood pressure measurement and systolic blood pressure <180 mmHg | • Pregnant patients or patients suspected of being pregnant |
| • Patients deemed unsuitable as participants by the investigator                   |                           |

CT, computed tomography; EVT, endovascular treatment; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; PAD, peripheral arterial disease; rt-PA, recombinant tissue-type plasminogen activator.

other staff will have access to the study arm and test results of the participants.

Intervention

For all patients, a manual blood pressure cuff will be placed around the lower leg or thigh of the unaffected side, and blood pressure will be measured while detecting the dorsalis pedis artery using an ultrasonic Doppler blood flow meter. The intervention (RIC) group will receive four cycles of 5 min of blood pressure cuff inflation, followed by 5 min of reperfusion. Cuff inflation in the RIC group will be set at 200 mmHg or 50 mmHg above the systolic blood pressure; however, if the patient cannot tolerate this, the cuff pressure may be reduced to 180 mmHg. This procedure will be performed once daily after enrollment, for a minimum of 3 days and a maximum of 7 days. In contrast, the control group will only undergo blood pressure measurements before and after the intervention period (the 40-min duration required for four cycles of RIC). Discomfort and pain will be assessed using a verbal rating scale ranging from 0 to 4 (0, no pain; 1, mild pain; 2, moderate pain; 3, severe pain; 4, very severe pain) (13).

Primary outcome

The primary outcome will be a good functional outcome at 3 months after stroke onset, with a target mRS score of 0–1, 0–2, and 0–3 in the mild, moderate, and severe groups, respectively. The mRS scores will be determined by face-to-face assessments or structured telephone interviews performed by various assessors, who will be blinded to the allocation results.

Secondary outcomes

The secondary outcomes will be the proportion of patients with good functional outcome (assessed by the mRS) in each group at 3 months after stroke onset; change in NIHSS scores from before RIC to after RIC; incidence of major adverse cardiovascular events (MACE), aspiration pneumonia, and all-cause mortality within 90 days after stroke onset; frequency of adverse RIC-related events; and proportion of patients with an mRS score of 0–1.

Data monitoring body

Our monitoring staff will be responsible for reviewing the various records of the study and confirming the enrollment status; research plan compliance; completeness, accuracy, and consistency of the data; and compliance with ethical guidelines. The monitoring staff will confirm the contents of the case report forms and ensure research integrity. In addition, the monitoring staff will oversee and manage the condition of each device. The monitoring staff will report any deviations from the protocol, its various procedures, and applicable regulatory requirements to the principal investigator and confirm that appropriate measures are taken and recorded in order to ensure that any identified deviations do not recur. The principal investigator agrees to cooperate with the monitoring staff to ensure that all issues discovered during such monitoring are handled and recorded.

Sample size calculation

The target sample size is based on previous reports (Table 3). In this study, patients will be divided into three groups based on their neurological severity at enrollment, and the functional outcome at 3 months after stroke onset will be determined for each group. A good functional outcome will be defined as mRS scores of 0–1, 0–2, and 0–3 for the mild (NIHSS score at enrollment, 5–9), moderate (NIHSS score at enrollment, 10–14), and severe (NIHSS score at enrollment, 15–20) groups, respectively. We hypothesize that the rates of good functional outcome are 35 and 50% in the control and RIC groups, respectively; accordingly, with 80% power at the 5% significant level, a sample size of 334 patients is necessary. Furthermore, to account for an expected drop-out rate of 10% (e.g., withdrawal of consent, lost to follow-up), we plan to enroll a total of 400 patients.
TABLE 2  Study procedures for eligible patients with acute ischemic stroke.

| Procedures/Data collection | Screening (days 0–1) | Registration/ Baseline visit 1 (day 1) | Visit 2 (day 3) | Visit 3 (day 7) | Visit 4 (day 30 or discharge) | Visit 5 (day 90 ± 14 days) |
|---------------------------|----------------------|--------------------------------------|----------------|----------------|--------------------------------|---------------------------|
| **Enrollment**            |                      |                                      |                |                |                                |                           |
| Informed consent          | ×                    |                                      |                |                |                                |                           |
| Demographic characteristics| ×                    |                                      |                |                |                                |                           |
| Confirmation of patient background and medical history | × |                                      |                |                |                                |                           |
| Randomization             | ×                    |                                      |                |                |                                |                           |
| **Intervention**          |                      |                                      |                |                |                                |                           |
| RIC                       | ×                    | ×                                    | ×              | ×              | ×                              |                           |
| **Assessment**            |                      |                                      |                |                |                                |                           |
| Concomitant medication confirmation | × | ×                                    | ×              | ×              | ×                              | ×                          |
| Physical examination (including height and weight) | × |                                      |                |                |                                |                           |
| Vital signs               | ×                    | ×                                    | ×              | ×              | ×                              | ×                          |
| Systolic blood pressure measurement of the upper and lower limbs | × | ×                                    | ×              | ×              |                                |                           |
| NIHSS                     | ×                    | ×                                    | ×              | ×              | ×                              | ×                          |
| mRS                       | ×                    |                                      |                |                |                                |                           |
| Questionnaire and pain scale | × | ×                                    | ×              | ×              | ×                              | ×                          |
| Description for the case report form | × | ×                                    | ×              | ×              | ×                              | ×                          |
| Confirmation of adverse events | × | ×                                    | ×              | ×              | ×                              | ×                          |
| Electrocardiogram         | ×                    |                                      |                |                |                                |                           |
| **Biomarkers**            |                      |                                      |                |                |                                |                           |
| Blood biochemistry        | ×                    |                                      |                |                |                                |                           |
| Brain CT or MRI scan      | ×                    |                                      |                |                |                                |                           |

CT, computed tomography; MRI, magnetic resonance imaging; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; RIC, remote ischemic conditioning.

Statistical analysis

The target populations for analysis will be defined as follows. The full analysis set (FAS) will comprise all randomized participants, with the exclusion of those who never received any protocol treatment, those who underwent at least one protocol treatment but whose post-protocol data are not available, and those who did not meet the eligibility criteria.
The intention-to-treat population will comprise all subjects who underwent randomization. The per-protocol set will comprise those in the full analysis set, with the exclusion of subjects with remote ischemia and poor conditioning compliance (<80%). The population for the safety analysis will comprise all subjects who received at least one protocol treatment.

Statistical analysis of the primary outcome

The primary outcome (a good outcome based on the mRS score at 90 days after stroke onset) will be reported as the percentage. Percentage differences between the RIC and control groups will be evaluated using the Chi-square test. Additionally, multiple regression analysis will be performed. For the multiple regression analysis, corresponding odds ratios and 95% confidence intervals will be calculated, and a P-value < 0.05 will be considered to be statistically significant.

Statistical analysis of secondary outcomes

Percentage differences between the RIC and control groups in terms of the incidence of MACE, aspiration pneumonia, frequency of adverse RIC-related events, and all-cause mortality within 90 days will be evaluated using the Chi-square test. The change in NIHSS score from the time of enrollment to 1 week later will be evaluated in a two-sided analysis of variance, with α = 0.05 and power = 0.80. Additionally, with stratified analysis, we will compare the differences in the achievement rate (percentage) of the primary outcome in the RIC and control groups according to the severity of the disease at the time of enrollment (mild, moderate, and severe groups), age (dichotomized by the median of all enrolled patients), sex, presence of occlusion or severe stenosis of the main cerebral artery, treatment with rt-PA and/or EVT, and presence of diabetes mellitus.

Discussion

Recently, clinical studies of RIC in patients with AIS are attracting a lot of attention. Indeed, several clinical studies are ongoing. The ongoing study by Purroy et al. is a study of acute stroke within 8 h of symptom onset with mRS 0–2 at 90 days as the primary outcome (25). The study of Blauenfeldt et al. is also an ongoing study of acute stroke within 4 h of symptom onset with mRS at 3 months as the primary outcome (26). To our knowledge, our study will be the first clinical trial to investigate the effect of RIC after ischemic stroke in Japan. RIC is safe, feasible, and offers similar
clinical benefits to exercise therapy after stroke, while being less physically taxing for the patient (27, 28). This trial may help determine whether RIC should be recommended as a routine clinical strategy for patients with ischemic stroke. RIC may then become one of the choice for therapy in most patients with ischemic stroke. Regarding the pathophysiology for RIC, three potential mechanisms have been proposed: humoral, immunoregulatory, and neurotrophic factors. In addition, several studies indicate that the underlying protective mechanisms of remote ischemic conditioning are associated with its ability to attenuate production of free radicals, promote the cell survival pathway, modulate the immune system, or inhibit the apoptotic cell signaling pathways (29–33). However, these proposed theories still require confirmation.

To date, several clinical studies have evaluated the effectiveness of RIC in patients with acute ischemic stroke, and no study has confirmed its efficacy except for a small clinical trial. Recently, three published clinical studies reported that RIC performed in four cycles of 5 min of inflation and 5 min of deflation for only 1 day after stroke onset did not significantly reduce the brain infarct volume growth or improve the functional outcome at 3 months (10–12). Furthermore, a recent report by Landman et al. studies acute ischemic stroke within 24 h of onset with infarct size on day 4 after admission. In this study, RIC was repeated twice daily with at least 6 h in between and continued for the duration of hospitalization for a maximum of 4 days. However, on average, RIC was conducted for only 2 days and this study did not show significant reduction of the brain infarct size (34). One reason for the inconsistency regarding RIC’s effectiveness may be the lack of an established RIC protocol. Previous study by An et al. demonstrated a favorable effect of RIC on the clinical outcome at 3 months using an RIC protocol of five cycles of cuff inflation (to 180 mmHg for 5 min) and deflation (for 3 min) twice per day throughout the duration of the hospitalization (mean, 11.2 days; range, 8–14 days) although this is very small study (35). Additionally, two published experimental studies found that repeated RIC for 14 consecutive days was associated with a smaller infarct size in an animal model for brain ischemia (36, 37). Of these two studies, the study by Ren et al. showed that a single episode of RIC afforded short-term protection including reduction of brain infarct size, while brain infarct size was further ameliorated when combined with repeated RIC during the 14 days after reperfusion (36). Furthermore, a recent animal model study reported that the number and duration of the cycles determined the efficacy of RIC (38). Specifically, two cycles of RIC were insufficient to decrease infarct size, whereas four, six, and eight cycles decreased the infarct size. Similarly, cycles with 2 or 5 min of ischemia decreased the infarct size, whereas cycles with 10 min of ischemia did not reduce the infarct size. In previous experimental studies, three to five 5-min cycles of RIC reduced the infarct size and improved the neurological deficit (33, 39, 40). Our previous experimental study also showed that four cycles of RIC, with each occlusion and release phase lasting 5 min, had efficacy in reducing the infarct volume (41). Based on these reports, it may be essential to perform RIC for a suitable number of cycles and consecutive days to achieve a good functional outcome. Therefore, we will perform four cycles of RIC, alternating 5 min of inflation and 5 min of deflation, repeated over 3–7 days.

The primary outcome of this trial will be good functional outcome at 3 months, determined by the mRS score. In most previous studies, the definition of a good functional outcome at 3 months (based on the mRS) was the same for all patients, regardless of the NIHSS score at stroke onset. Our study differs from previous studies in this respect. Referring to previous studies showing the relationship between NIHSS at admission and mRS at discharge or 3 months after stroke onset, our study will divide patients into three groups based on their neurological severity (assessed by the NIHSS) at enrollment into the trial and will use different target mRS scores to define a good outcome at 3 months according to the severity group. For the safety outcome, the analysis will be based on the entire randomized sample. This approach has been used in a recent comparative study and is considered reasonable given the excellent safety profile of RIC.

Finally, we aim to include 400 patients. In this study, the criteria were simplified to enroll a more significant number of patients with various types of strokes. Therefore, given that ~200 patients with ischemic stroke are admitted to our hospital each year, and since this is a multicenter study, we expect to be able to recruit 400 patients during a recruitment period of 2 years.

Summary and conclusions

RIC-AIS part two is a multicenter, prospective, randomized, open-label, parallel-group clinical trial designed to evaluate the efficacy of RIC in patients with acute ischemic stroke. RIC may contribute to improving the functional prognosis by reducing inflammation, oxidative stress, and cerebral edema mediated by humoral, immunoregulatory, and neurotrophic factors. This trial may help determine whether RIC should be recommended as a routine clinical strategy for patients with acute ischemic stroke.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Tokyo Women’s
Medical University Hospital (approval number: 2021-0136). The patients/participants provided their written informed consent to participate in this study.

Author contributions

KKit was the chief investigator and a guarantor. KKit, KI, THo, and ST comprised the steering committees. YS performed the statistical analysis. MSAi supervised the data management center. YY, KT, MSak, TO, KM, SH, YH, HY, KKKim, THi, YI, TK, RD, TM, and MH were the study investigators. All authors contributed to the article and approved the submitted version.

Funding

This study was supported in part by the Mihara Cerebrovascular Disorders Research Promotion Fund. The funding source had no role in the design or conduct of the study.

References

1. Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthexene Y, Cheng R, et al. MRI-guided thrombolysis for stroke with unknown time of onset. *N Engl J Med.* (2018) 379:611–22. doi: 10.1056/NEJMoai804355

2. Goyal M, Menon BK, van Zwan WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet.* (2016) 387:1723–31. doi: 10.1016/S0140-6736(16)00163-X

3. Toyoda K, Inoue M, Koga M. Small but steady steps in stroke medicine in Japan. *J Am Heart Assoc.* (2019) 8:e013306. doi: 10.1161/JAHA.119.013306

4. Kobayashi S, Fukuma S, Ilenoue T, Fukuhara S. Effect of edaravone on neurological symptoms in real-world patients with acute ischemic stroke. *Stroke.* (2019) 50:1805–11. doi: 10.1161/STROKEAHA.118.024351

5. Lee H, Yun HJ, Ding Y. Timing is everything. Exercise therapy and remote ischemic conditioning for acute stroke patients. *Brain Circ.* (2021) 7:178–86. doi: 10.4033/bc.bc.35.21

6. Zhao W, Li S, Ren C, Meng R, Jin K, Ji X. Remote ischemic conditioning for stroke: clinical data, challenges, and future directions. *Ann Clin Transl Neurol.* (2019) 6:186–96. doi: 10.1002/actn.3691

7. Baig S, Moyle B, Nair KPS, Redgrave J, Ali A. Remote ischemic conditioning for stroke: unanswered questions and future directions. *Stroke Vasc Neurol.* (2021) 6:298–309. doi: 10.1136/scan.2020-000722

8. Zhao W, Yu W, Li S, Ren C, Ji X. Remote ischemic conditioning for stroke: where we are and where to go. *Conduit Med.* (2021) 4:185–91.

9. Hess DC, Blaunefeldt RA, Andersen G, Hougaard KD, Hoda MN, Ding Y, et al. Remote ischemic conditioning: a new paradigm of self-protection in the brain. *Nat Rev Neurol.* (2015) 11:698–710. doi: 10.1038/nrneurol.2015.223

10. Pico F, Lapergue B, Ferrigno M, Rosso C, Meseguer E, Chadenat ML, et al. Effect of in-hospital remote ischemic preconditioning on brain infarct growth and clinical outcomes in patients with acute ischemic stroke: THE RESCUE BRAIN randomized clinical trial. *JAMA Neurol.* (2020) 77:725–34. doi: 10.1001/jama-neurol.2020.0326

11. Hougaard KD, Hjort N, Ziedler D, Sørensen L, Nargaard A, Hansen TM, et al. Remote ischemic preconditioning as an adjunct therapy to thrombolysis in patients with acute ischemic stroke: a randomized trial. *Stroke.* (2014) 45:159–67. doi: 10.1161/STROKEAHA.113.001346

12. England TJ, Hedstrom A, O’Sullivan S, Donnelly R, Barrett DA, Sarmad S, et al. RECASYT (Remote Ischemic Conditioning After Stroke Trial): A pilot randomized placebo controlled phase II trial in acute ischemic stroke. *Stroke.* (2017) 48:1418–25. doi: 10.1161/STROKEAHA.116.016429

13. Khadka J, Schonwevel PG, Pesudovs K. Comparing the measurement properties of visual analogue and verbal rating scales. *Ophthalmic Physiol Opt.* (2022) 42:205–17. doi: 10.1111/opo.12917

14. Lees KR, Zivin JA, Ashwood T, Davalos A, Davis SM, Diener HC, et al. NXY-059 for acute ischemic stroke. *N Engl J Med.* (2006) 354:588–600. doi: 10.1056/NEJMoa052980

15. Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, et al. NXY-059 for the treatment of acute ischemic stroke. *N Engl J Med.* (2007) 357:562–71. doi: 10.1056/NEJMoa070240

16. Shuaib A, Bornstein NM, Diener HC, Dillon W, Fisher M, Hammer MD, et al. Partial aortic occlusion for cerebral perfusion augmentation: safety and efficacy of NeuroFlo in Acute Ischemic Stroke trial. *Stroke.* (2011) 42:1680–90. doi: 10.1161/STROKEAHA.110.609933

17. Ginsberg MD, Palesch YJ, Hill MD, Martin RH, Moy CS, Barsan WG, et al. High-dose albumin treatment for acute ischaemic stroke (ALLAS) Part 2: a randomised, double-blind, phase 3, placebo-controlled trial. *Lancet Neurol.* (2013) 12:1049–58. doi: 10.1016/S1474-4422(13)70223-0

18. Hess DC, Wechslser LR, Clark WM, Savitz SL, Ford GA, Chiu D, et al. Safety and efficacy of multipotent adult progenitor cell injection in acute ischemic stroke (MASTERS): a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Neurol.* (2017) 16:360–8. doi: 10.1016/S1474-4422(17)30046-7

19. Saver JL, Starkman S, Eckstein M, Stratton SJ, Pratt FD, Hamilton S, et al. Prehospital use of magnesium sulfate as neuroprotection in acute stroke. *N Engl J Med.* (2015) 372:528–36. doi: 10.1056/NEJMoai408827

20. Elkins J, Velkamp R, Montaner J, Johnston SC, Singhal AB, Becker K, et al. Safety and efficacy of natalizumab in patients with acute ischaemic stroke (ACTION): a randomised, placebo-controlled, double-blind phase 2 trial. *Lancet Neurol.* (2017) 16:217–26. doi: 10.1016/S1474-4422(16)30357-X

21. Kimura K, Kuriyama M, Inoue M, Tsujimoto K, Kagami M. Relationships between NIHSS score and outcome at discharge in ischemic stroke patients admitted within 3 hours of onset. *Ipn J Stroke.* (2003) 25:312–21. doi: 10.3995/2003.25.312
22. Hill MD, Goyal M, Menon BK, Nogueira RG, McGtaggart RA, Demchuk AM, et al. Efficacy and safety of nimodipine for the treatment of acute ischemic stroke (ESCAPE-NA1): a multicentre, double-blind, randomised controlled trial. Lancet. (2020) 395:878–87. doi: 10.1016/S0140-6736(20)30258-0

23. Nogueira RG, Jadhav AP, Haussen DC, Bonefa A, Budzik RF, Bhuya P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. N Engl J Med. (2018) 378:11–21. doi: 10.1056/NEJMoa1706442

24. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. N Engl J Med. (2018) 378:708–18. doi: 10.1056/NEJMoa1713973

25. Parry F, Argeu G, Mauri G, García-Vázquez C, Vicente-Pascual M, Pereira C, et al. Remote ischemic preconditioning among acute ischemic stroke patients in catalonia: REMOTE-CAT PROJECT. Front Neurol. (2020) 11:569696. doi: 10.3389/fneur.2020.569696

26. Blauenfeldt RA, Hjort N, Gude MF, Behrndt A, Fisher M, Valentin JB, et al. A multicentre, randomised, sham-controlled trial on remote ischemic conditioning in patients with acute stroke (RESIST): rationale and study design. Eur Stroke J. (2020) 5:94–101. doi: 10.1177/2396987319884408

27. Wang Q, Wills M, Han Z, Geng X, Ding Y. Mini Review (Part I): An experimental concept on exercise and ischemic conditioning in stroke rehabilitation. Brain Circ. (2020) 6:242–7. doi: 10.4103/bc.bc_63_20

28. Wills M, Ding Y. Mini-review (Part II): A clinical consideration on exercise and ischemic conditioning in stroke rehabilitation. Brain Circ. (2021) 7:225–9. doi: 10.4103/bc.bc_56_21

29. Wang YJ, Shen J, Gao Q, Ye ZG, Yang SY, Liang HW, et al. Ischemic postconditioning protects against global cerebral ischemia/reperfusion-induced injury in rats. Stroke. (2008) 39:983–90. doi: 10.1161/STROKEAHA.107.499079

30. Xing B, Chen H, Zhang M, Zhao D, Jiang R, Liu X, et al. Ischemic postconditioning inhibits apoptosis after focal cerebral ischemia/reperfusion injury in the rat. Stroke. (2008) 39:2362–9. doi: 10.1161/STROKEAHA.107.507939

31. Zhao H, Saposzyk RM, Steinberg GK. Interrupting reperfusion as a stroke therapy: ischemic postconditioning reduces infarct size after focal ischemia in rats. J Cereb Blood Flow Metab. (2006) 26:1114–21. doi: 10.1038/jcbfm.9600348

32. Zhao H. Ischemic postconditioning as a novel avenue to protect against brain injury after stroke. J Cereb Blood Flow Metab. (2009) 29:873–85. doi: 10.1038/jcbfm.2009.13

33. Hoda MN, Siddiqui S, Herberg S, Perysamy-Thanavasan S, Bhatia K, Hafez SS, et al. Remote ischemic postconditioning is effective alone and in combination with intravenous tissue-type plasminogen activator in murine model of embolic stroke. Stroke. (2012) 43:2794–9. doi: 10.1161/STROKEAHA.112.60373

34. Landman TR, Schoon Y, Warle MC, Meijer FJ, Leeuw FE, Thijssen DH. The effect of repeated remote ischemic postconditioning after an ischemic stroke (REPOST): a randomized controlled trial. Int J Stroke. (2022) 14:17474930221104710. doi: 10.1177/17474930221104710

35. An JQ, Cheng YW, Guo YC, Wei M, Gong MJ, Tang YL, et al. Safety and efficacy of remote ischemic postconditioning after thrombolysis in patients with stroke. Neurology. (2020) 95:e355–63. doi: 10.1212/WNL.0000000000008884

36. Ren C, Wang P, Wang B, Li N, Li W, Zhang C, et al. Limb remote ischemic per-conditioning in combination with post-conditioning reduces brain damage and promotes neuroglobin expression in the rat brain after ischemic stroke. Restor Neurol Neurosci. (2015) 33:369–79. doi: 10.3233/RNN-140413

37. Doepner TR, Zechmeister B, Kaltwasser B, Jin F, Zheng X, Majid A, et al. Very delayed remote ischemic post-conditioning sustains sustained neurological recovery by mechanisms involving enhanced angiogenesis and peripheral immunosuppression reversal. Front Cell Neurosci. (2018) 12:383. doi: 10.3389/fncel.2018.00383

38. Johnsen J, Pryds K, Salman R, Løfgren B, Kristiansen SB, Bøtker HE. The remote ischemic preconditioning algorithm: effect of number of cycles, cycle duration and effector organ mass on efficacy of protection. Basic Res Cardiol. (2016) 111:10. doi: 10.1007/s00395-016-0529-6

39. Sun J, Tong L, Luan Q, Deng J, Li Y, Li Z, et al. Protective effect of delayed remote limb ischemic postconditioning: role of mitochondrial K(ATP) channels in a rat model of focal cerebral ischemia/reperfusion injury. J Cereb Blood Flow Metab. (2012) 32:851–9. doi: 10.1038/jcbfm.2011.199

40. Hu S, Dong H, Zhang H, Wang S, Hou L, Chen S, et al. Noninvasive limb remote ischemic preconditioning contributes neuroprotective effects via activation of adenosine A1 receptor and redox status after transient focal cerebral ischemia in rats. Brain Res. (2012) 1459:81–90. doi: 10.1016/j.brainres.2012.04.017

41. Kitagawa K, Saitoh M, Ishizuka K, Shimizu N. Remote ischemic conditioning during cerebral ischemia reduces infarct size through enhanced collateral circulation in murine focal cerebral ischemia. J Stroke Cerebrovasc Dis. (2018) 27:831–8. doi: 10.1016/j.jstrokecerebrovasdis.2017.09.068