

Remote Ischemic Conditioning After Stroke Trial 2: A Phase IIb Randomized Controlled Trial in Hyperacute Stroke

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Background—Repeated episodes of limb ischemia and reperfusion (remote ischemic conditioning [RIC]) may protect the brain from ischemic reperfusion injury.

Methods and Results—We performed a phase IIb blinded dose-escalation sham-controlled trial in patients with hyperacute stroke, randomized 1:1 to receive RIC (four 5-minute cycles) or sham to the nonparetic upper limb, in 3 blocks of increasing dose, starting within 6 hours of ictus. The primary outcome was trial feasibility (recruitment, attrition). Secondary outcomes included adherence, tolerability, safety (serious adverse events), plasma biomarkers at days 1 and 4 (S100- β protein, matrix metalloproteinase-9, and neuron-specific enolase), and functional outcome. Sixty participants were recruited from 2 centers (3 per month) with no loss to follow-up: time to randomization 4 hours 5 minutes (SD 72 minutes), age 72 years (12), men 60%, blood pressure 154/80 mm Hg (25/12), National Institutes of Health Stroke Scale 8.4 (6.9), and 55% thrombolysed. RIC was well tolerated with adherence not differing between RIC and sham, falling in both groups on day 3 ($P=0.001$, repeated measures ANOVA) because of discharge or transfer. S100 β increased in the sham group (mean rise 111 pg/mL [302], $P=0.041$, repeated measures ANCOVA) but not the RIC group. There were no differences in matrix metalloproteinase-9, neuron-specific enolase, number with serious adverse events (RIC 10 versus sham 10, $P=0.81$), deaths (2 versus 4, $P=0.36$), or modified Rankin Scale score (2 [interquartile range 1–4], 2 [interquartile range, 1–3]; $P=0.85$).

Conclusions—RIC in hyperacute stroke is feasible when given twice daily for 2 days and appears safe in a small population with hyperacute stroke. A larger phase III trial is warranted.

Clinical Trial Registration—URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT02779712. (*J Am Heart Assoc.* 2019;8:e013572. DOI: 10.1161/JAHA.119.013572.)

Key Words: feasibility • randomized controlled trial • remote ischemic conditioning • stroke

Remote ischemic conditioning (RIC) uses repeated cycles of transient limb ischemia and reperfusion to induce

organ protection from ischemic reperfusion injury and tolerance to subsequent ischemic events. The mechanisms underlying RIC are not fully understood but have been attributed to neurohumoral pathways linking the pre-conditioned tissue to the brain, resulting in early and late windows of protection.^{1,2}

Experimentally, RIC applied before (pre-conditioning), during (per-conditioning), and after (post-conditioning) an ischemic stroke decreases cerebral inflammation and edema and reduces apoptosis in the ischemic penumbra through inhibition of the mitochondrial permeability transition pore.^{3–5} Administration of a protein synthesis inhibitor, afferent nerve blocker or K_{ATP} channel antagonist attenuates the neuroprotective effects of RIC.^{4,6} In a meta-analysis of preclinical stroke, remote pre-, per-, and post-conditioning reduced infarct volume by 35%, was effective in permanent and transient models of ischemia, and improved neurological outcome.⁷

Five proof-of-concept randomized clinical trials in stroke and RIC have been published: in populations before carotid stenting (pre-conditioning),⁸ during acute ischemic stroke

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Clinical Perspective

What Is New?

- This is the first randomized controlled trial in patients with hyperacute ischemic stroke to test an increasing dose of remote ischemic conditioning. Repeated dosing until day 2 was feasible in terms of adherence, and the dosing regimen for larger remote ischemic conditioning trials should consider this alongside local patient pathways.

What Are the Clinical Implications?

- Beneficial clinical signals exist from several small pilot and proof-of-concept studies using remote ischemic conditioning after stroke and these findings warrant further testing in a well-designed, larger phase III trial.

(per-conditioning),^{9,10} and after ischemic stroke caused by intracranial stenosis (post-conditioning).^{11,12} In RECAST-1 (Remote Ischemic Conditioning After Stroke Trial), we demonstrated excellent intervention tolerability in patients with acute stroke.¹⁰ Although limited by a small sample size, there was also a significant decrease in National Institutes of Health Stroke Scale (NIHSS) score at day 90 and augmentation of neuroprotective proteins, plasma heat shock protein-27 (HSP27) and phosphorylated HSP27, in the RIC group.¹³

In the current study, we aimed to demonstrate feasibility and safety of increasing doses of remote ischemic per-conditioning in patients presenting to the hospital with hyperacute stroke.

Methods

Trial Design

RECAST-2 (Remote Ischemic Conditioning After Stroke Trial 2), was a 2-center, feasibility, dose-escalation, outcome-blinded, randomized, placebo-controlled trial. The trial was conducted in accordance with the Declaration of Helsinki and the International Conference of Harmonisation of Good Clinical Practice, sponsored by the University of Nottingham (United Kingdom), received authorization from the local research ethics committee (West Midlands, April 15, 2016), and was a registered clinical trial (NCT02779712).

Patients

Adults 18 years and older were invited to participate if they had a clinical stroke with onset in the past 6 hours. Exclusion criteria were premorbid dependency (modified Rankin Scale [mRS] score >3), dementia, Glasgow Coma Scale score <8, malignancy, pregnancy, and significant comorbidities. We did not mandate that baseline neuroimaging (as part of standard

care) was required before randomization, but, in practice, the brain scan and approaching the participant for the trial occurred in parallel, with the result available before trial inclusion. The trial recruited patients from University Hospitals of Derby and Burton (UHDB) NHS Foundation Trust and Nottingham University Hospitals (NUH) NHS Trust in the United Kingdom between August 2016 and April 2018. UHDB and NUH receive ≈900 and 1300 patients with strokes per year, respectively. Consent was obtained by the research practitioner from each patient or legal representative if the patient was unable to consent. Clinical and safety assessments were performed at baseline (prerandomization), day 4 (face-to-face), and day 90 (telephone).

Intervention

RIC was performed by trained trial staff immediately after randomization and included 4 cycles of intermittent limb ischemia: alternating 5 minutes of inflation (20 mm Hg above systolic blood pressure [BP]) followed by 5 minutes of deflation of a standard upper arm BP cuff in the nonparetic arm. The control group received a sham procedure mimicking the intervention protocol, but cuff inflation only reached 30 mm Hg. The intervention was performed manually using a standard BP cuff. Preclinically, repeated RIC cycles are more effective than a single set of cycles¹⁴; hence, we increased the dose in 3 phases: the first 20 participants received 1 “dose” of RIC/sham, ie 4 cycles of cuff inflation and deflation; participants 21 to 40 received a second dose (4 cycles of RIC/sham) 1 hour after the first dose; and the final 20 participants (41–60) were also administered twice-daily dosing starting the following morning up to and including day 4 (total 8 doses). Delivery time of each cycle (seconds) and reasons for discontinuation were recorded.

Primary Outcome

The primary outcome was trial feasibility describing recruitment rate, time to recruitment, number recruited per center, and attrition to follow-up.

Secondary Outcomes

Tolerability

Tolerability of increasing doses of RIC: duration cuff tolerated, number of cycles, adherence to RIC, and reasons for poor adherence.

Clinical measures and safety

Clinical secondary outcomes included safety: vascular events (recurrent stroke, myocardial infarction, limb ischemia, and venous thromboembolism), death, neurological deterioration

(increase in NIHSS ≥ 4 points), neurovascular limb damage, and tissue injury. Comparison of serious adverse events (SAEs) by treatment with thrombolysis provided further assessment of safety in this subgroup. Function was assessed at day 90 by telephone interview blinded to treatment allocation: dependency (mRS),¹⁵ disability (Barthel Index),¹⁶ Zung depression scale,¹⁷ quality of life (EuroQol-5D), and cognition (Modified Telephone Interview for Cognitive Status [TICS-M]).¹⁸

Laboratory measures

Immediately before treatment and on day 4 (± 1), blood samples were collected for: (1) surrogate markers of brain injury, which might be attenuated if RIC improves ischemic reperfusion injury (plasma S100 β protein, matrix metalloproteinase [MMP-9], neuron-specific enolase [NSE], by multiplex technology, Merck Millipore Ltd); S-100 β , NSE, and MMP-9 also act as surrogate markers of infarct volume and prognosis in acute ischemic stroke^{19,20}; and (2) HSP27 (DouSet ELISA, R&D Systems), which is a biomarker implicated in the mechanisms of ischemic conditioning¹⁰ and is neuroprotective in experimental stroke.¹³ Assays and analysis of data were performed blinded to treatment allocation.

Sample Size

No formal power calculation was performed for this feasibility study. Considering resources, competing trials and time,

recruiting 60 participants from 2 centers was deemed an appropriate number, at an anticipated rate of recruitment of 1.5 recruits per center per month, to inform a larger trial on application of RIC in the hyperacute setting in terms of trial feasibility and increasing RIC dose.

Randomization and Blinding

Participants were recruited using web-based randomization with computerized minimization distributing the patients on a 1:1 ratio into RIC or sham groups. Minimization variables were age (≥ 70 years), sex (male), NIHSS (≥ 10), and systolic BP (≥ 160 mm Hg). The research practitioner delivering the intervention could not be blinded. Adjudicated SAEs and outcomes, day 90 interview, laboratory measures, and statistical analyses were performed blinded to treatment allocation.

Statistical Analysis

Baseline characteristics and functional outcomes of RIC and control groups were compared using chi-square or Fisher exact tests for binary data; continuous data were compared using *t* test or Mann–Whitney *U* tests; and recurrent clinical events were compared using hazard ratios and univariate Cox regression analyses (SPSS version 24, IBM). Additionally, day 90 mRS was compared using ordinal logistic regression.

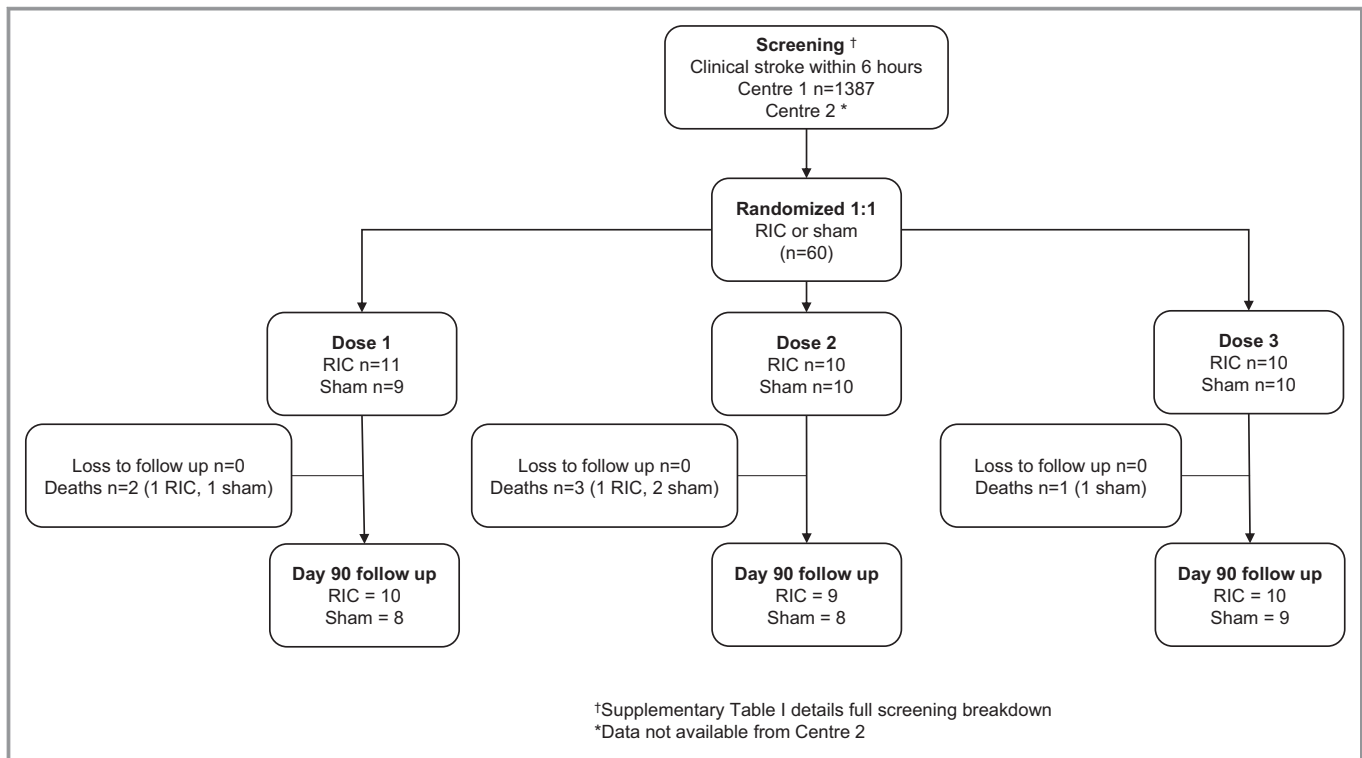


Figure 1. Trial flow. RIC indicates remote ischemic conditioning.

Table 1. Baseline Characteristics

Characteristic	RIC	Sham
No.	31	29
Age, y (SD)*	70.9 (13.4)	73.7 (10.2)
Men*	21 (70)	15 (50.0)
BP, mm Hg		
Systolic*	146 (24)	162 (23)
Diastolic	78 (12)	83 (11)
Heart rate	77 (13)	80 (18)
Admission ECG in AF	11 (36.7)	12 (40.0)
NIHSS*	6 [3–9]	7 [3–12]
GCS	15 [14–15]	15 [14–15]
Premorbid mRS	0 [0–2]	0 [0–1]
Stroke to randomization, min	254 [254–343]	199 [149–261]
Admission to randomization, min	195 [174–277]	93 [66–168]
Thrombolized	16 (51.6)	17 (58.6)
Mechanical thrombectomy	0 (0)	0 (0)
Final diagnosis†		
Ischemic stroke	28 (90.3)	27 (93.1)
TIA	2 (6.5)	2 (6.9)
Hemorrhagic stroke	0 (0)	0 (0)
Clinical syndrome		
Total anterior circulation	6 (20.0)	5 (16.7)
Partial anterior circulation	9 (30.0)	14 (46.7)
Lacunar	9 (30.0)	8 (26.7)
Posterior circulation	4 (13.3)	0 (0.0)
Medical history		
Hypertension	14 (46.7)	11 (36.7)
Diabetes mellitus	10 (33.3)	2 (6.7)
Known AF	12 (40.0)	10 (33.3)
Hyperlipidemia	14 (46.7)	9 (30.0)
Stroke	9 (30.0)	5 (16.7)
TIA	6 (20.0)	2 (6.7)
Ischemic heart disease	5 (16.7)	4 (13.3)
Peripheral vascular disease	1 (3.3)	1 (3.3)

Data are expressed as mean values (SD), median [interquartile range], or number (percentage). AF indicates atrial fibrillation; BP, blood pressure; GCS, Glasgow Coma Scale; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; TIA, transient ischemic attack.

*Minimization variables.

†One participant was diagnosed with functional disorder in the remote ischemic conditioning (RIC) group.

Repeated measures ANOVA with no covariate adjustment compared adherence to treatment between groups. Repeated measures ANCOVA, adjusting for baseline NIHSS, compared plasma biomarkers taken on day 1 and day 4, with further adjustment using Sidak multiple comparisons test (SPSS

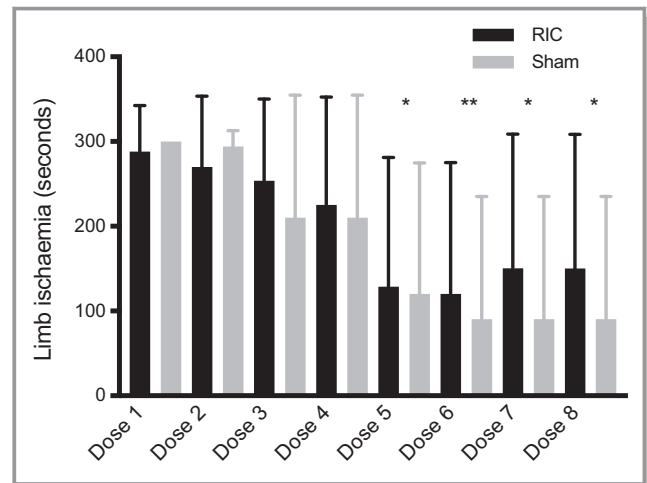


Figure 2. Adherence to remote ischemic conditioning (RIC) or sham by dose number and mean total duration of limb ischemia (seconds±SD). Maximum length of cuff inflation is 300 seconds per dose (4×5 minutes per cycle). Compared with dose 1, there is a significant fall in adherence over time from day 3 (* $P=0.001$, ** $P<0.001$ repeated measures ANOVA), with no between-group differences ($P=0.64$). “n” sham/RIC=dose 1: 29/31; dose 2: 19/21; and doses 3 to 8: 10/10.

version 24 and Prism 7 for Mac OS X version 7.0c). Associations between S100 β and functional outcome were tested using Pearson’s correlation coefficient. Subgroup analyses were not performed at a dose level since numbers were considered too small. Data in the figures are mean±SD unless otherwise stated. Statistical significance was taken at $P<0.05$.

Data Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Patients

The trial commenced in August 2016, completed follow-up in August 2018, and enrolled 60 participants (31 and 29 in the RIC and sham groups, respectively; 20 per dose block) (Figure 1). The mean age of all participants was 72 years (SD 12), 60% were men, mean BP was 154/80 mm Hg (SD 25/12 mm Hg), and median NIHSS was 6 (interquartile range, 3–11). There were no baseline statistical differences between groups for age, sex, and baseline stroke severity (Table 1). The RIC group was randomized later (254 versus 195 minutes, $P=0.003$), contained more participants with diabetes mellitus (33% versus 7%, $P=0.02$), and had a lower mean systolic BP

(146 versus 162 mm Hg, $P=0.01$). The final diagnosis was ischemic stroke in 55 cases (92%), transient ischemic attack in 4 cases (7%), and functional disorder in 1 case (2%).

Trial Feasibility

The recruitment rate averaged 1.5 participants per center per month ($n=20$ center 1, $n=40$ center 2). The main reasons for exclusion included presentation >6 hours from onset of symptoms (42.5%), presenting outside of working hours (13.4%), and nonstroke diagnoses (12.5%) (Table S1). The median time to randomization was 255 minutes (interquartile range, 186–298), with 33 (55%) receiving thrombolysis. There were no losses to follow-up. The sham appeared feasible since when asked at day 90 which intervention patients received, 56 (93%) did not know, 2 (4%) were incorrect, and 2 were (4%) correct.

Compliance

RIC was well tolerated with no statistical differences between RIC and sham regarding duration of cuff inflation (Figure 2).

Table 2. Summary of Secondary Clinical Outcomes and SAEs

SAE	RIC (n=31)	Sham (n=29)	HR (95% CI)	P Value
No with SAE				
Any SAE	10 (32.3)	10 (34.5)	0.81 (0.33–1.96)	0.81
Fatal	2 (6.5)	4 (13.8)	0.46 (0.8–2.5)	0.36
All stroke and ND*				
Extension/recurrent ischemic stroke	2 (6.5)	6 (20.7)	0.28 (0.06–1.37)	0.12
Symptomatic HTI	2 (6.5)	1 (3.4)	1.85 (0.17–20.38)	0.62
Early ND	1 (3.2)	0 (0.0)
Seizure	0 (0.0)	1 (3.4)
TIA	1 (3.2)	0 (0.0)
MI	0 (0.0)	0 (0.0)
VTE				
PE	0 (0.0)	1 (3.4)
DVT	0 (0.0)	0 (0.0)

Analyses performed using unadjusted Cox regression. DVT indicates deep vein thrombosis; HR, hazard ratio; MI, myocardial infarction; PE, pulmonary embolism; SAE, serious adverse event; TIA, transient ischemic attack; VTE, venous thromboembolism. *One participant in the sham group had neurological deterioration (ND) and hemorrhagic transformation of infarction (HTI), and 1 participant in the remote ischemic conditioning (RIC) group had HTI and recurrent stroke (only the first event is counted in regression analyses).

Adherence was high in the first 2 days but there was a significant fall on day 3 (dose 5) in the RIC and sham groups to 40% and 43%, respectively ($P=0.001$, repeated measures ANOVA), with no between-group differences ($P=0.64$) secondary to either early discharge or the participant moving to another rehabilitation setting. In the first 48 hours, procedure intolerance in the RIC group (cuff pressure intolerance, headache, agitation) leading to incomplete treatment of 4 cycles occurred in 5 of 62 (8%) offered doses (Table S2).

Safety and Clinical Outcomes

There was no difference in the number of participants with an SAE (Table 2 and Table S3) and no episodes of limb ischemia or injury. The mortality rate was 10% with 4 deaths in the sham group ($n=1$ extension/recurrent ischemic stroke, $n=1$ hemorrhagic transformation of infarction, $n=1$ early neurological deterioration, $n=1$ gradual decline) and 2 in the RIC group ($n=1$ hemorrhagic transformation of infarction, $n=1$ malignancy). Extension and recurrent ischemic stroke were more frequent in the sham group (6 versus 2 events) (unadjusted hazard ratio, 0.28; 95% CI, 0.06–1.37 [$P=0.12$]). A total of 83% of recurrent cerebrovascular events occurred in the first 48 hours. RIC appeared to be safely administered in the thrombolized cohort, with no differences in SAEs between groups (Table S4).

Laboratory Measures

Plasma S100 β increased significantly in the sham group from day 1 to day 4, mean difference, 111 pg/mL (95% CI, 5.6–216 [$P=0.041$], repeated measures ANOVA, adjusted for baseline NIHSS) (Figure 3). No differences in plasma S100 β were present in the RIC group between days 1 and 4 (mean difference, 27.5 pg/mL; 95% CI, –14.5 to 69.5 [$P=0.187$]) nor were there significant differences between groups at day 4 (adjusted $P=0.35$). Day 4 plasma S100 β correlated significantly with baseline NIHSS (Pearson's correlation coefficient, $r=0.561$; $P<0.001$) and day 90 mRS ($r=0.41$, $P=0.006$). MMP-9 concentration was nonsignificantly higher in the sham group compared with the RIC group (change from baseline, mean difference, 15.3 ng/mL [95% CI, –2.6 to 33.2], $P=0.09$). There were no differences between groups with respect to NSE. Heat shock protein 27 assays proved unreliable and data are not presented.

Functional Outcomes

There were no significant differences between groups with respect to functional outcomes, although the trial was not large enough to detect these (Table 3 and Figure 4). Telephone data collection at day 90 was feasible for measures

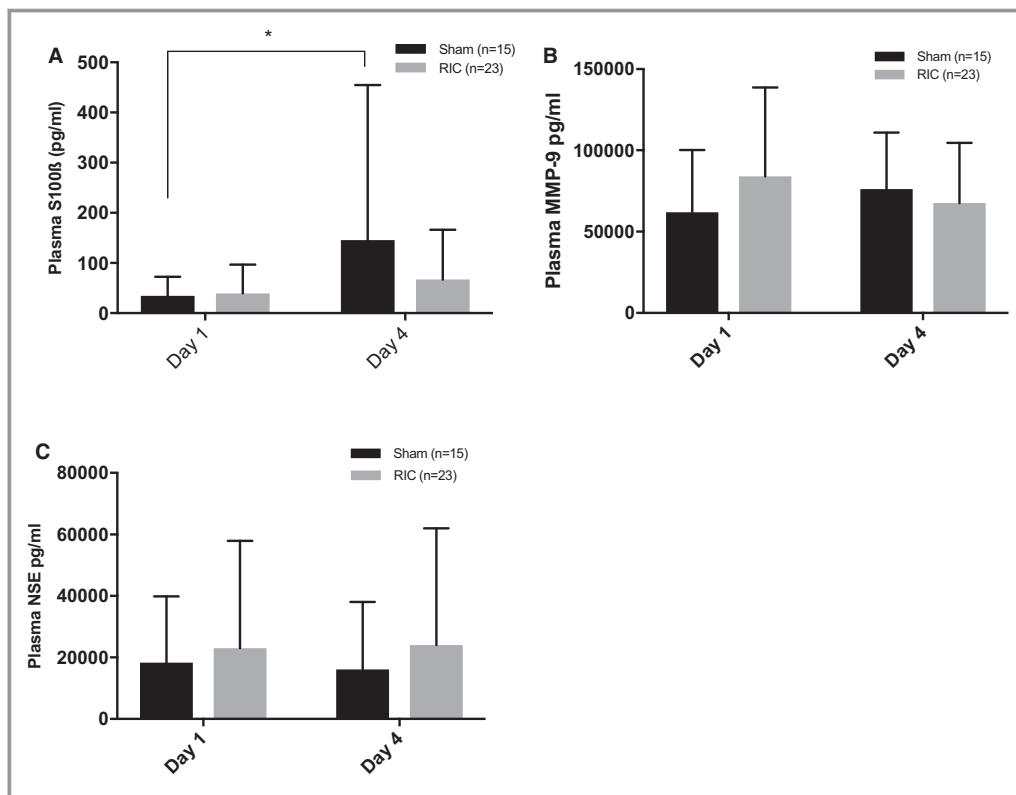


Figure 3. Plasma S100β (A), matrix metalloproteinase-9 (MMP-9, B), and neuron-specific enolase (NSE, C) on days 1 and 4 by treatment group. S100β levels increase by day 4 in the sham group from 34.5 pg/mL (SD 37.8) to 145.6 pg/mL (309.1), mean difference 111 pg/mL (95% CI, 5.6–216; $P=0.041^*$). There were no significant between-group differences at day 4. Analysis by repeated measures ANCOVA, Sidak correction for multiple comparisons, and adjusted for baseline stroke severity. RIC indicates remote ischemic conditioning.

of dependency (mRS), disability (Barthel index, BI), mood (Zung), cognition (TICS-M), and quality of life (EuroQoL), similar to other large trials.²¹

Discussion

RECAST-2 has demonstrated the feasibility of conducting a randomized controlled trial of remote ischemic per-conditioning in hyperacute stroke across 2 centers in terms of recruitment, intervention delivery, attrition, compliance of increasing dose to day 2, and use of an effective sham.

RECAST-2 is the first stroke and RIC trial to evaluate alternative dosing strategies. Overall, the optimal dosing and method of application of RIC in stroke remains unclear. There is noticeable heterogeneity in completed and ongoing clinical trials ranging from daily administration using both arms in post-conditioning secondary prevention studies (cuff pressure to 200 mm Hg),^{11,12} to single lower limb application using cuff pressures 120 mm Hg above the systolic BP in acute ischemic stroke.²² Strategies appear to be based on the population studied rather than from information provided by

preclinical data. Importantly, an experimental dose-finding study in postconditioned stroke rats determined that 3 cycles of 5/5 minutes limb ischemia and reperfusion was more effective than 15/15 seconds and 8/8 minutes.⁴ Previous trials have delivered RIC daily for up to 300 days,^{11,12} initiated in the subacute phase after stroke. It is therefore feasible to deliver RIC for a prolonged period using an automated machine. We chose the maximum dose to stop at day 4 since this covers the hyperacute phase and prolonged effects of the treatment are anticipated.²³ We also expected it would not be possible to administer RIC using a manual BP cuff for longer than this, which proved to be the case. In RECAST-2, repeated dosing until day 2 was feasible in terms of adherence, and the dosing regimen for larger RIC trials should consider this alongside local patient pathways. The main reason for treatment discontinuation was not cuff pressure intolerance but transfer of the participant to a different setting or discharge home.

The absence of any SAEs relating to limb ischemia or injury, especially in the thrombolized cohort, is reassuring. The safety of RIC in hyperacute stroke, however, requires

Table 3. Functional Outcome by Group at Day 4 and Day 90

Functional Measure	RIC	Sham	P Value
Day 4*	n=30	n=24	
NIHSS	6.4 (9.4)	9.5 (12.8)	0.30
Change NIHSS	-3.0 (3.8)	-1.4 (6.3)	0.35
Day 90	n=31	n=29	
mRS (/6)			
Median	2 [1-4]	2 [1-3]	0.85
mRS 3 to 6	12 (40)	14 (46.6)	0.46
Barthel Index (/100)	100 [65-100]	100 [57.5-100]	0.89
Zung depression score [†]	46.25 [33.75-53.75]	42.5 [37.5-52.5]	0.94
EuroQoL HUI [†]	0.514 (0.377)	0.482 (0.393)	0.77
EuroQoL VAS [†]	70.8 (23.0)	69.8 (19.2)	0.87
TICS-M [†]	23 [20-25]	23.5 [21-27]	0.89

Data are expressed as mean (SD), median [interquartile range], or number (percentage). Imputed value for death: Barthel index -5; National Institutes of Health Stroke Scale (NIHSS) 42. mRS indicates modified Rankin Scale.

*Day 4 NIHSS—sham n=24, remote ischemic conditioning (RIC) n=30 (data missing attributable to early discharge or refused). Analyzed by independent *t* test, Mann-Whitney *U* test, or chi-square test as appropriate.

[†]Number for EuroQoL Health Utilities Index (HUI): 24 (sham)/28 (RIC), EuroQoL visual analogue scale (VAS) 22/24, Zung 17/16, and Modified Telephone Interview for Cognitive Status (TICS-M) 14/14. Number reduced by: (1) carers answering on behalf of participants who could not respond (n=17), (2) refused to answer questions on mood and cognition, and (3) death (n=6).

further evaluation since this is a small population. RIC has potential antiplatelet effects,²⁴ which may be beneficial in ischemic stroke, but could exacerbate hemorrhagic

transformation of infarction or lead to deterioration if administered in intracerebral hemorrhage before confirmation of the diagnosis. One pre-hospital RIC trial, however, reported no clinical deterioration in 37 participants with primary intracerebral haemorrhage.⁹

The majority of recurrent cerebrovascular events occurred within the first 48 hours, reflecting early ischemic reperfusion injury, which can manifest clinically as recurrent ischemia, hemorrhagic transformation of infarction, cerebral edema, and expansion of the original infarct. The trial was not powered to detect reductions in these events, but we observed tendency in favor of RIC towards reduced risk of recurrent fatal and nonfatal stroke. In addition, there are biochemical signals of efficacy evidenced by increased plasma biomarkers of brain injury (S100 β) in the placebo group not seen in the RIC group. S100 β is a recognized surrogate marker of infarct volume and functional outcome,¹⁹ and in this study correlated significantly with baseline stroke severity and day 90 mRS.

It is recognized that RIC leads to an immediate period of ischemic tolerance lasting 1 to 2 hours, followed by a second window of protection 12 to 24 hours later, lasting 48 to 72 hours.²⁵ Preclinically, alteplase combined with RIC has an additive effect²⁶ and a single dose of RIC can have long-lasting protective effects for up to 6 days.²³ Further, the time window of RIC application in experimental models extends up to 6 hours post-ictus²⁷ and combining per- and post-conditioning may tackle both early and late phases of ischemic reperfusion injury. Per-conditioning was more effective at reducing infarct volume than a pre-conditioning stimulus in one study²⁸ but this difference is not borne out

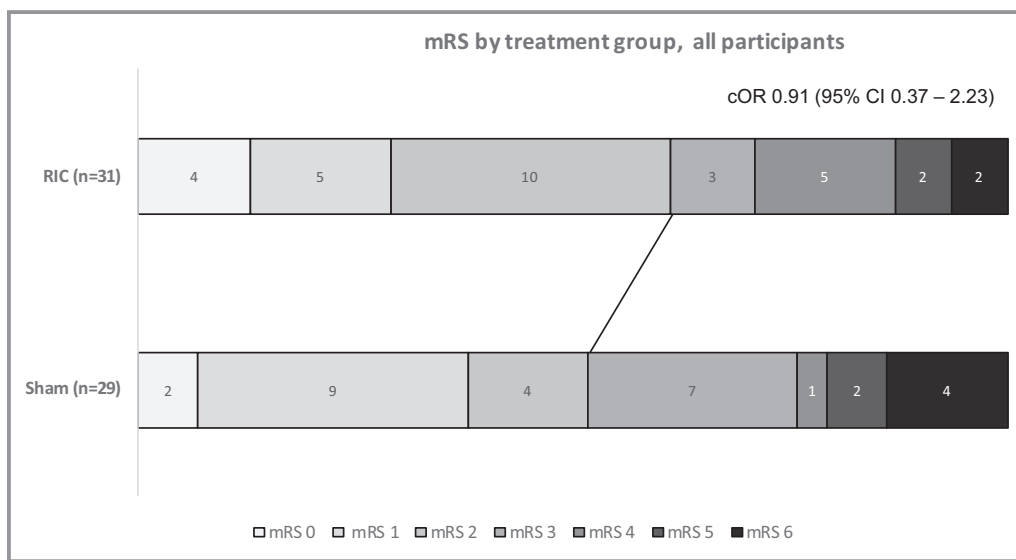


Figure 4. Day 90 modified Rankin Scale (mRS) score by treatment group. Unadjusted common odds ratios (cORs) and 95% CIs comparing groups are analyzed by ordinal logistic regression. There was no significant interaction when treatment*thrombolysis was introduced into the model. The line demarcates dichotomy at functional independence, an mRS of ≤ 2 . RIC indicates remote ischemic conditioning.

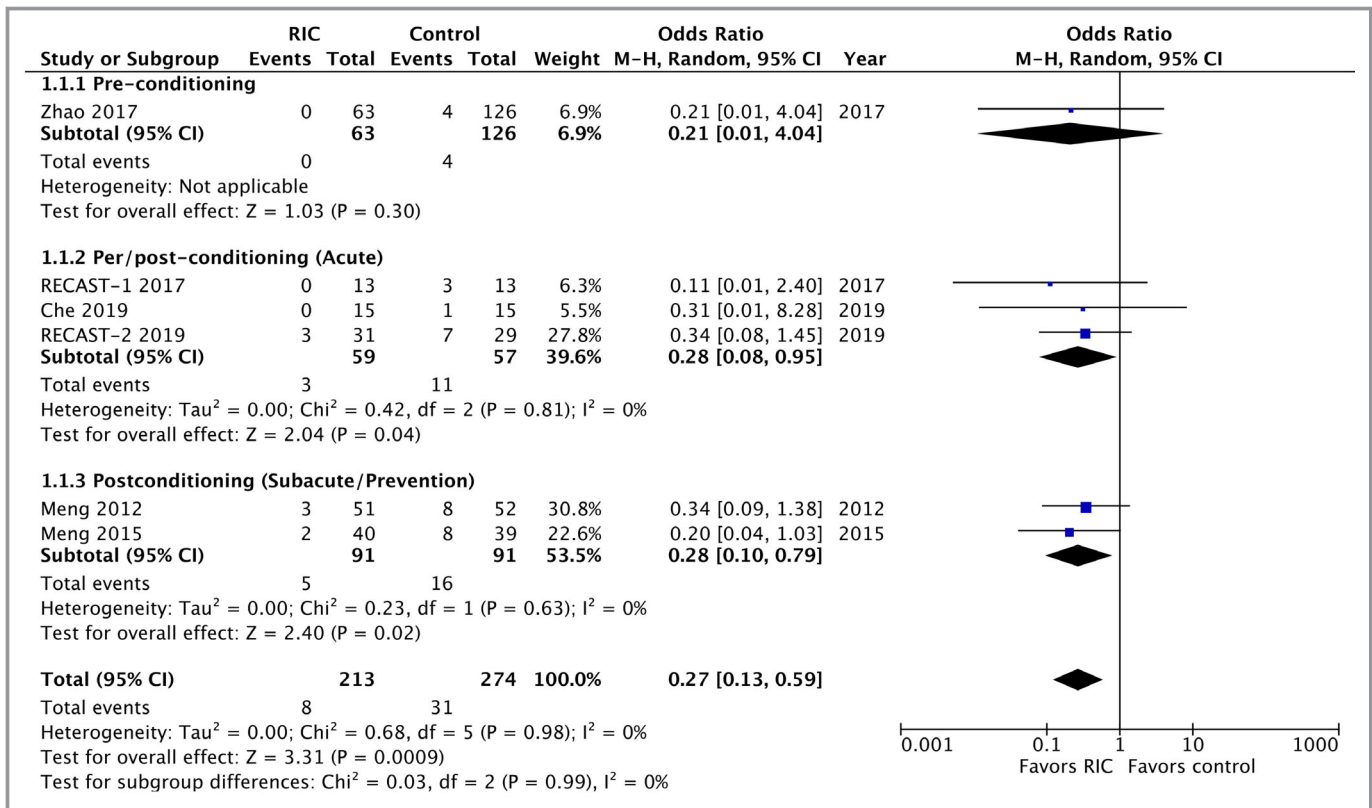


Figure 5. Recurrent vascular events (nonfatal and fatal stroke, nonfatal and fatal myocardial infarction) in randomized controlled trials assessing remote ischemic conditioning (RIC) in stroke. RECAST indicates Remote Ischemic Conditioning After Stroke Trial; Ref, reference number.

in meta-analysis of experimental data.⁷ Since strokes are difficult to predict, pre-conditioning is a viable strategy in acute ischemia, whereas pre-conditioning may be more suited towards high-risk populations, eg, before carotid intervention⁸ or after a transient ischemic attack.

A recent Cochrane Review exploring RIC for preventing and treating ischemic stroke has highlighted the paucity of published randomized clinical trials in this area.²⁹ Interestingly, recurrence in ischemic stroke (by end of trial) was significantly reduced.^{8,11,12,29} In updating the meta-analysis with results from RECAST-1,¹⁰ RECAST-2, and Che et al,³⁰ and organizing groups into pre-, per-, and post-conditioning trials, RIC significantly reduces the composite outcome of recurrent vascular events (odds ratio, 0.27; 95% CI, 0.13–0.59) (Figure 5).^{8,10–12,30} This is consistent with secondary analyses in the cardiac literature (RIC and acute myocardial infarction) where recurrent cardiovascular and cerebrovascular events were reduced by half.³¹ It is not intuitive that brief periods of RIC can lead to protection from vascular events at much later time points (and repeated doses may be required) but the finding deserves further exploration in clinical trials.

Study Limitations

RECAST-2 was a high-quality randomized trial strengthened by assessment of multiple doses, concealment of allocation, and blinded outcome assessments. Limitations include the inability to blind the investigator who performed the intervention, potentially introducing bias into RIC/sham compliance. Second, small sample size, which is sufficient to answer questions of feasibility, introduces risk that other findings may be due to chance, especially since there was an imbalance in systolic BP and diabetes mellitus between groups at baseline. A larger randomized trial is needed to further evaluate efficacy and safety. Third, no participants undergoing mechanical thrombectomy were included (as a result of the need to deliver RIC manually and logistics of transfer to another site). We are unable to comment on the safety of RIC in mechanical thrombectomy but RIC seems feasible and safe in an observational study of mechanical thrombectomy.³² Finally, because of a limited budget, we did not perform any mechanistic neuroimaging studies that determine recanalization or reperfusion rates; however, whilst use of RIC in acute ischemic stroke seems most

likely to benefit patients with ischemic reperfusion injury, there is suggestion that in patients with persisting occlusion, RIC may still reduce infarct risk.⁹

Conclusions

RIC in hyperacute ischemic stroke is feasible, appears safe, and can be administered in repeated doses reliably for 2 days. It is an attractive prospect since it bears low cost and would be simple to administer. A larger phase III trial is warranted.

Acknowledgments

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England designed the trial, analyzed the data, and wrote the article. Hedstrom and Jackson screened for participants, acquired the data, and helped revise the article. O'Sullivan processed and analyzed blood biomarkers and revised the article. Woodhouse provided statistical support. Sprigg and Bath contributed to trial design and management, and revised the article.

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Disclosures

Bath is Stroke Association Professor of Stroke Medicine and is an NIHR Senior Investigator. The remaining authors have no disclosures to report.

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SUPPLEMENTAL MATERIAL

Table S1. Reasons for exclusion from centre 1.

Reason for exclusion	N = 1387	(%)
Greater than 6 hours since onset of symptoms	575	41.5
Premorbid dependency mRS \geq 4	12	0.9
Dementia	34	2.5
Coma – GCS <8	3	0.2
Malignancy or significant co-morbidity thought to limit life expectancy	12	0.9
Blood glucose < 3.5mmol/L	0	0.0
Pregnancy	0	0.0
Out of hours	186	13.4
Non stroke	174	12.5
No-one to assent	8	0.6
Trial on hold	9	0.6
Poor prognosis/ Died	11	0.8
Recruited	20	1.4
Refused	7	0.5
Out of area	1	0.1
No English	2	0.1
Competing trial	13	0.9
Anaphylactic reaction to thrombolysis	2	0.1
Resolved minor stroke	8	0.6

TIA	119	8.6
ICH	170	12.3
Thrombectomy	3	0.2
Seizures/ vomiting at presentation	1	0.1
Researcher unavailable (e.g. annual leave, training, sick leave)	17	1.2

(Data not available from centre 2, which were discarded before analysis due to changes in European law on data protection (General Data Protection Regulation (GDPR), see <https://eugdpr.org>).

Table S2. Reasons for non-compliance.

RIC group	
Dose 1	n=1 refused all cycles [†]
Dose 2	n=1 refused all cycles [†] , n=4 reduced compliance (1=cuff pressure, 1=headache, 2=cannula)
Dose 3	n=1 weekend (no researcher available to administer intervention), n=1 relative refusal, n=1 cuff pressure
Dose 4	n=1 agitated, refused remaining doses; n=1 weekend; n=1 relative refused remaining doses; n=1 cuff pressure
Dose 5	n=2 weekend, n=1 relative refusal, n=1 felt unwell on cuff release, remainder discharged
Dose 6	n=2 weekend, n=1 refused (previously felt unwell with cuff release), n=1 relative refused, remainder discharged
Dose 7	n=1 refusal, n=1 relative refusal, remainder discharged
Dose 8	n=1 refusal, n=1 relative refusal, remainder discharged

Sham	
Dose 2	n=1 deteriorated during treatment, n=1 relative refused all doses after dose 1, n=1 weekend
Doses 3&4	n=1 weekend (no researcher available to administer intervention)

† one participant refused doses 1 and 2 but was fully compliant doses 3-8	
Early discharge explains the remainder of non-compliance in the sham group	

Table S3. Serious adverse events.

Treatment Group	Trial number	Thrombo-lysis	Time post randomisation (days d, hours hr, minutes m)	Adjudicated Diagnosis	Day of death	Relationship
Sham	11	Y	0 d, 5 hr, 58 m	Extension/recurrent ischaemic stroke	3	Improbable
	11	Y	0 d, 6 hr, 33 m	Pneumonia		Improbable
	13	N	0 d, 15 hr, 7 m	Extension/recurrent ischaemic stroke		Improbable
	13	N	0 d, 0 hr, 2 m	Haematemesis		Improbable
	13	N	3 d, 12 hr, 17 m	Pulmonary embolism		Improbable
	15	N	0 d, 9 hr, 54 m	Extension/recurrent ischaemic stroke		Improbable
	23	N	0 d, 0 hr, 36 m	Extension/recurrent ischaemic stroke		Improbable
	23	N	1 d, 21 hr, 56 m	Symptomatic Haemorrhagic transformation of infarct	6	Possible
	28	Y	0 d, 3 hr, 29 m	Seizure / convulsions		Improbable
	28	Y	0 d, 0 hr, 1 m	Early neurological deterioration	4	Improbable
		Y	0 d, 22 hr, 19 m	Asymptomatic Haemorrhagic transformation of infarct		Possible
	41	Y	1 d, 22 hr, 20 m	Pneumonia		Improbable
	38	N	15 d, 22 hr, 39 m	Traumatic rectus sheath haematoma		Improbable
	38	N	6 d, 0 hr, 30 m	Urinary tract infection		Improbable
	41	Y	12 d, 23 hr, 11 m	Pneumonia		Improbable
	41	Y	31 d, 1 hr, 35 m	Urinary tract infection		Improbable
	41	Y	36 d, 19 hr, 35 m	Complication of original stroke	47	Improbable
	47	Y	0 d, 2 hr, 25 m	Extension/recurrent ischaemic stroke		Improbable

	53	Y	0 d, 5 hr, 46 m	Extension/recurrent ischaemic stroke		Improbable
RIC	9	Y	0 d, 21 hr, 7 m	Extension/recurrent ischaemic stroke		Improbable
	12	Y	0 d, 17 hr, 32 m	Symptomatic Haemorrhagic transformation of infarct	2	Possible
	16	N	2 d, 6 hr, 40 m	Urinary tract infection		Improbable
	22	Y	0 d, 0 hr, 0 m	Fever, undetermined source		Improbable
	39	N	7 d, 10 hr, 10 m	Symptomatic Haemorrhagic transformation of infarct		Possible
	39	N	1 d, 22 hr, 6 m	Lung Malignancy	30	Improbable
	39	N	21 d, 10 hr, 10 m	Recurrent ischaemic stroke		Improbable
	33	N	17 d, 17 hr, 55 m	Pneumonia		Improbable
	42	Y	2 d, 7 hr, 37 m	Urinary tract infection		Improbable
	35	Y	9 d, 20 hr, 5 m	Urinary tract infection		Improbable
	52	N	2 d, 23 hr, 23 m	Transient ischaemic attack		Improbable
	44	N	6 d, 19 hr, 55 m	Pneumonia		Improbable

Table S4. Serious Adverse Events and Clinical outcomes by thrombolysis.

Serious Adverse Event	RIC	Sham	p
Thrombolysed			
Number	16	17	
No with SAE			
Any SAE	5 (31.3)	5 (29.4)	1.0
Fatal	1 (6.2)	3 (17.6)	0.60
All stroke and ND*			
Extension/recurrent ischaemic stroke	1 (6.2)	3 (17.6)	0.60
Symptomatic HTI	1 (6.2)	0	0.49
Early ND	0	1 (5.9)	1.0
Seizure	0	1 (5.9)	1.0
Limb injury	0	0	-
Not thrombolysed			
Number	15	12	
No with SAE			
Any SAE	5 (33.3)	5 (41.7)	1.0

Fatal	1 (6.7)	1 (8.3)	1.0
All stroke and ND*			
Extension/recurrent ischaemic stroke	1 (6.7)	3 (25)	0.29
Symptomatic HTI	1 (6.7)	1 (8.3)	1.0
Early ND	0	0	-
Seizure	0	0	-
Limb injury	0	0	-

Post hoc analyses, performed using 2-sided Fisher's Exact test. Data are number (%)

SAE, serious adverse event, ND neurological deterioration, HTI haemorrhagic transformation of infarction