

ORIGINAL RESEARCH



Remote Ischemic Conditioning in Patients With Acute Ischemic Stroke Ineligible for Recanalization Therapies: A Phase II Trial

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BACKGROUND: Remote ischemic conditioning (RIC) has shown neuroprotective potential in preclinical stroke models. In patients with acute ischemic stroke, trials report heterogeneous results, supporting ongoing debate about clinical efficacy, especially in those ineligible for recanalization. This trial assessed RIC benefit in this population and evaluated HSP27 (heat shock protein 27) and HIF-1 α (hypoxia-inducible factor 1- α) as biomarkers of RIC-related biological activity.

METHODS: This was a multicenter, phase II, proof-of-concept, block-randomized, controlled, parallel-arm, PROBE (Prospective Randomized Open Blinded End Point)-designed clinical trial. Patients with acute ischemic stroke (National Institutes of Health Stroke Scale [NIHSS] score of 5–25) who were ineligible for recanalization therapies were randomized 1:1 within 9 hours of symptom onset to RIC (4 5-minute cycles of upper-arm ischemia/reperfusion) plus standard medical therapy or standard therapy alone. The primary outcome was the percentage change in NIHSS score at 72 hours. Secondary outcomes included NIHSS score changes at 24 and 48 hours, 90-day modified Rankin Scale, tolerability, and changes in plasma HSP27 and HIF-1 α concentrations from 24 to 72 hours.

RESULTS: Eighty patients (40 per group) were enrolled across 4 centers from August 2021 to March 2024. Although slightly in favor of RIC treatment, median NIHSS score percentage change at 72 hours did not differ between the RIC and control groups (23.6% versus 29.9%; adjusted coefficient, -3.8 [95% CI, -19.7 to 12.1]; $P=0.635$). No significant differences in NIHSS score percentage change were observed at 24 or 48 hours. Ninety-day poor functional outcome (modified Rankin Scale score, 3–6) was similar between groups (57.5% versus 65.0%; adjusted odds ratio, 0.79 [95% CI, 0.21 – 2.97]; $P=0.732$). RIC was completed by 97.5% of patients with minimal treatment-related discomfort. No major adverse events occurred. No between-group differences were detected in HIF-1 α or HSP27 changes from 24 to 72 hours.

CONCLUSIONS: In patients with acute ischemic stroke ineligible for recanalization therapies, early RIC was safe, feasible, and well tolerated but did not improve neurological outcomes or modify HIF-1 α and HSP27 levels.

REGISTRATION: URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT04400981.

GRAPHIC ABSTRACT: A [graphic abstract](#) is available for this article.

Key Words: acute ischemic stroke ■ biomarkers ■ ischemia ■ neuroprotection ■ reperfusion

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Nonstandard Abbreviations and Acronyms

EPO	erythropoietin
ERK	extracellular signal-regulated kinase
HIF-1α	hypoxia-inducible factor 1-alpha
HSP	heat shock protein
HSP27	heat shock protein 27
HSPB1	heat shock protein beta-1
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
nNOS	neuronal NO synthase
PROBE	Prospective Randomized Open Blinded End Point
RIC	remote ischemic conditioning
SITS-MOST	Safe Implementation of Thrombolysis in Stroke—Monitoring Study
VEGF	vascular endothelial growth factor

CLINICAL PERSPECTIVE

What Is New?

- In this multicenter phase II PROBE trial (Prospective Randomized Open Blinded End Point), remote ischemic conditioning, delivered as four 5-minute cycles of upper-arm ischemia/reperfusion within 9 hours of stroke onset, in patients with acute ischemic stroke ineligible for intravenous thrombolysis or mechanical thrombectomy was safe and highly feasible but did not improve early neurological recovery (National Institutes of Health Stroke Scale score at 24–72 hours), 90-day functional outcomes, or circulating HIF-1 α (hypoxia-inducible factor 1-alpha) and HSP27 (heat shock protein 27) profiles compared with standard medical therapy alone.

What Are the Clinical Implications?

- These findings suggest that remote ischemic conditioning is unlikely to provide clinically meaningful neuroprotection in moderate-to-severe patients with acute ischemic stroke ineligible for recanalization therapies, underscoring the need to refine conditioning intensity and duration, optimize timing, and incorporate biomarker-guided approaches in future translational trials.
- While confirming the safety and practicality of remote ischemic conditioning in the hyperacute setting, this trial highlights the persistent translational gap between promising preclinical neuroprotection and neutral clinical results, informing the design of next-generation trials targeting patients without access to recanalization therapies.

Remote ischemic conditioning (RIC) is a promising neuroprotective strategy that involves applying brief, nonlethal ischemic episodes to a distant organ or tissue, thereby conferring protection against subsequent ischemic brain injury. RIC is increasingly being explored as a treatment for ischemic stroke, with the aim of reducing brain injury and improving neurological outcomes. Its neuroprotective effects are thought to arise from the activation of systemic and cellular pathways that ultimately shield neurons from ischemic damage. A major pathway involves HSPs (heat shock proteins), which enhance protein stability, prevent apoptosis, and promote cell survival.^{1–4} In addition, RIC induces HIF-1 α (hypoxia-inducible factor 1-alpha), which modulates the inflammatory response and improves cerebral blood flow^{5–7} in experimental stroke models. Despite growing interest in biochemical mechanisms associated with RIC, previous clinical trials in patients with stroke have not systematically evaluated biomarkers of treatment response. Exceptions include trials^{8,9} reporting inconclusive findings regarding HSP27 (heat shock protein 27) as a reliable indicator of RIC-induced biological activity as well as biomarker-focused studies^{10–12} whose results remain heterogeneous, underscoring the persistent uncertainty regarding the identification of reliable, reproducible, and clinically meaningful biomarkers of treatment response.

Numerous preclinical studies have shown the efficacy of RIC in experimental stroke models, showing reduction in infarct volume, improvements in neurological function, and enhancements in long-term recovery.^{13–17} These findings have prompted translation into clinical research. In patients with acute ischemic stroke, RIC has consistently been shown to be well tolerated and safe.^{8,9,18–21} The procedure, typically involving cyclic cuff inflation on a limb to induce transient ischemia, is generally associated with minimal discomfort. Despite this favorable safety profile, evidence regarding the clinical efficacy of RIC in improving neurological outcome has been mixed. Several randomized controlled trials have assessed RIC as an adjunct to intravenous thrombolysis or mechanical thrombectomy, but the results have been inconsistent.^{9,18–22} Nonetheless, given the encouraging preclinical data, interest persists in patients who are not eligible for reperfusion therapies. To date, however, clinical evidence remains limited. The observed increase in the likelihood of excellent functional outcomes at 90 days in patients with moderate ischemic stroke is primarily supported by the RICAMIS study (Remote Ischemic Conditioning for Acute Moderate Ischemic Stroke),²³ whereas the improvement in disability at 3 months is reported in the REMOTE-CAT trial (REMOTE Ischemic Perconditioning Among Acute Ischemic Stroke Patients in Catalonia),²¹ highlighting the need for further investigation.

Taking a deliberately translational approach, this phase II exploratory, proof-of-concept trial had a dual objective: to evaluate the preliminary efficacy signal of RIC in

patients with ischemic stroke within 9 hours of symptom onset who are not eligible for recanalization therapies, and to investigate whether HSP27 and HIF-1 α could serve as measurable biomarkers of RIC-related biological activity.

METHODS

Data Availability

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

Trial Design

The TRICS-9 (Trial on Remote Ischemic Conditioning in Acute Ischemic Stroke Within 9 Hours of Onset in Patients Ineligible to Recanalization Therapies) is a phase II, exploratory, proof-of-concept, multicenter, controlled, block-randomized, parallel-arm, open-label, interventional clinical trial comparing RIC plus standard medical therapy to standard medical therapy alone, in patients with acute ischemic stroke within 9 hours of onset who are not eligible for intravenous thrombolysis or mechanical thrombectomy. The study has a PROBE (Prospective Randomized Open Blinded End Point)²⁴ design. The Consolidated Standards of Reporting Trials 2025 guidelines were followed for reporting trial protocol and findings (URL: <https://www.consort-spirit.org/>).

The trial protocol was approved by the institutional ethical committee at each participating site. Written informed consent was obtained from enrolled patients or their legal representatives. If the patient was unable to provide consent and no representative was available, an emergency consent procedure was applied in compliance with European Union (EU) Regulation 536/2014 (section 36). In this situation, the patient's next of kin was informed whenever possible, and deferred written consent from the patient or his/her legal representative was then obtained as soon as the clinical condition allowed. Patient assent was explored in all cases. The first draft of the article was written by the first author, with contributions from all coauthors. The authors had unrestricted access to the data. Data extraction was performed by an independent data-management team from the Clinical Research Office at the University of Milano Bicocca. Statistical analyses were conducted by an independent statistician. Safety and trial continuation decisions were made based on recommendations from an independent Data and Safety Monitoring Board, which included clinical scientists, statisticians, and a stroke patient representative from Associazione per la Lotta all'Ictus Cerebrale. Details regarding inclusion and exclusion criteria, interventions, and assessments have been previously published.²⁵ The trial protocol and statistical analysis plan are available on clinicaltrials.gov.

Initially, the study protocol included only patients with anterior circulation strokes; however, it was later revised to also enroll patients with posterior circulation strokes due to slow recruitment with the goal of maximizing patient enrollment. Patients were eligible for inclusion in the trial if they had evidence of acute ischemic stroke of the anterior or posterior circulation due to either small or large vessel occlusion within 9 hours of symptom onset, age of 18 years or older, a score

on National Institutes of Health Stroke Scale (NIHSS; scores range from 0 to 42, with higher scores indicating a more severe deficit) score of 5 to 25, a prestroke of 0 to 2 on the modified Rankin Scale (mRS; ranging from 0 to 6, with a score of 0 indicating no disability and higher scores indicating more severe disability), and the patients or their surrogate had provided informed consent. Patients with stroke symptoms on awakening were eligible for enrollment if the time elapsed between the midpoint of sleep (calculated based on the estimated time of falling asleep and waking up) and randomization was ≤ 9 hours; patients with unknown time of onset were enrolled if the time elapsed since the patient was last known to be well was ≤ 9 hours. Patients eligible for either or both intravenous thrombolysis and mechanical thrombectomy were excluded from the trial. Other exclusion criteria were the evidence of intracranial hemorrhage, vascular malformation or intracranial masses on brain computed tomography or magnetic resonance imaging; rapidly improving neurological symptoms at the time of first evaluation; transient ischemic attack with symptom resolution at the time of first evaluation; amputation of the upper nonparetic arm, ulcers, phlebitis, or bad skin condition of the upper limbs; history of peripheral artery disease or sickle cell disease.

Randomization

The trial was conducted at 4 Italian academic hospitals equipped with comprehensive stroke centers. Participants were randomly assigned, in a 1:1 ratio, to RIC plus standard medical therapy (the RIC group) or to standard medical therapy alone (the control group). Randomization was performed with the use of a central, Web-based procedure, as per a computer-generated block randomization schedule stratified by center, to balance the 2 treatment groups.

Procedures

The experimental procedure took place in the Emergency Department immediately after randomization. A standard blood pressure cuff was applied to the nonparetic arm. Patients assigned to the RIC group underwent RIC by 4 cycles of manually induced upper limb ischemia, with 5 minutes of inflation (20 mmHg above systolic blood pressure) followed by 5 minutes of deflation. In the control group, the cuff was applied to the nonparetic arm for 40 minutes without inflating. All patients received standard care based on the investigator's discretion and existing guidelines.

Outcomes

Assessment of clinical outcomes was performed by a neurologist blinded to treatment allocation.

The primary efficacy outcome was early neurological improvement at 72 hours, defined as percent change in NIHSS score ($[\text{admission NIHSS score} - \text{NIHSS}_{72} \text{ score}] \times 100 / \text{admission NIHSS score}$). This metric was prespecified to account for baseline stroke severity heterogeneity and to provide a more sensitive continuous measure of neurological change in a phase II trial setting. Three secondary efficacy outcomes were assessed: NIHSS score percent change at 24 hours ($[\text{admission NIHSS score} - \text{NIHSS}_{24} \text{ score}] \times 100 / \text{admission NIHSS score}$); NIHSS score percent change at

48 hours $[(\text{admission NIHSS score} - \text{NIHSS}_{48} \text{ score}) \times 100 / \text{admission NIHSS score}]$; disability at 3 months, which was assessed using the mRS through in-person interviews with patients and caregivers, or via telephone interviews with either or both patients and caregivers when in-person assessments were not feasible.

The primary safety outcome was symptomatic intracerebral hemorrhage per the SITS-MOST (Safe Implementation of Thrombolysis in Stroke—Monitoring Study) definition (ie, a local or remote type 2 parenchymal hemorrhage on imaging 22 to 36 hours after treatment or earlier if the imaging scan was performed due to clinical deterioration combined with a neurological deterioration of 4 NIHSS score points from baseline or from the lowest NIHSS score between baseline and 24 hours or leading to death within 24 hours; a grading of type 2 parenchymal hemorrhage for intracranial hemorrhage indicates a coagulum exceeding 30% of the infarct with substantial space occupation).

Other secondary outcomes included RIC feasibility, defined as the proportion of patients randomized to a treatment group that completed the experimental intervention; tolerability of RIC, defined as a score above 0 on the Wong-Baker Faces Pain Rating Scale early after the experimental procedure and at 72 hours; the median variation of blood levels of HIF-1 α and HSP27 between 24 and at 72 hours.

Assessment of Blood Biomarkers

The analysis of laboratory samples collected from each Center was conducted centrally by a biotechnologist who was unaware of the patients' treatment allocation.

Plasma levels of HIF-1 α and HSP27 were quantified by a colorimetric enzyme immunoassay ELISA kit (Human HIF-1A ELISA Kit, Thermo Fisher Scientific; Human HSPB1 [HSP beta-1] ELISA Kit, Thermo Fisher Scientific) following the manufacturer's instructions. The difference between HIF-1 α levels at 24 and 72 hours was named HIF-1 α Diff72 and represented in box plots. Similarly, the difference between plasma concentrations of HSP27 at 24 and 72 hours was expressed as HSP27 Diff72 and represented by box plots.

An increase in either or both HIF-1 α and HSP27 levels at 72 hours compared with 24 hours was supposed to reflect the peripheral vascular effect of RIC.

Statistical Analysis

Based on the reanalysis of data from the National Institute of Neurological Disorders and Stroke trial,²⁶ the control group showed a $15 \pm 35\%$ change in NIHSS score at 24 hours. Considering that the median percent change in untreated patients is expected to slightly increase and that the variability to decrease by 72 hours due to neurological stabilization, the sample size for the TRICS-9 was set at 80 patients (40 per arm), providing 80% power to detect a 20% between-group difference in median NIHSS score percent change at 72 hours (40% in the treatment group versus 20% in the control group), based on the Wilcoxon-Mann-Whitney U test (2-sided $\alpha=0.05$). This effect size was considered clinically meaningful and prespecified in the statistical analysis plan. The primary null hypothesis was that RIC does not improve early neurological recovery compared with standard therapy alone.

Baseline characteristics were summarized and compared between study groups. Categorical variables were reported as counts and percentages and compared using the χ^2 or Fisher exact test, as appropriate. Continuous variables were summarized as medians with interquartile ranges and compared using the Wilcoxon-Mann-Whitney U test. Time from symptom onset to randomization was reported as median (interquartile range).

For the primary outcome, patients with neurological worsening were assigned a percent change of 0; patients who died within 72 hours were assigned the last available percent change, and a percent change of 0 was assigned when only the admission NIHSS score was available. The primary outcome, percentage change in NIHSS score at 72 hours, was analyzed using the Wilcoxon-Mann-Whitney U test. A secondary analysis was conducted using a generalized linear model adjusted for prespecified covariates, including age, sex, cardiovascular risk factors, and presence of intracranial vessel occlusion, following the intention-to-treat principle. Early neurological improvement at 24 and 48 hours was analyzed similarly, first with unadjusted Wilcoxon-Mann-Whitney U test tests and then with adjusted generalized linear models.

mRS scores at 90 days were dichotomized as good functional outcome (mRS score 0–2) versus poor outcome (mRS score, 3–6). Group differences were assessed using the χ^2 test, and multivariable logistic regression adjusted for relevant covariates was applied for confirmatory analysis under the intention-to-treat principle.

The incidence of symptomatic intracerebral hemorrhage according to the SITS-MOST definition was calculated and compared between groups.

Wong-Baker Faces Pain Rating Scale scores (<https://wongbakerfaces.org/>) were dichotomized as 0 versus >0 . Early posttreatment differences were assessed using the χ^2 test. Logistic regression was not feasible for early Wong-Baker Faces Pain Rating Scale due to zero events in the control group; at 72 hours, unadjusted logistic regression was used to compare groups.

Differences between 24- and 72-hour measurements of circulating levels of HIF-1 α and plasma HSP27s were analyzed using the Wilcoxon test and represented in box plots. A secondary analysis using a generalized linear model adjusted for age, sex, and cardiovascular risk factors was also performed according to the intention-to-treat principle. Missing data were handled using complete-case analysis for adjusted models. In addition, multiple imputation was performed for the main outcomes using models including all relevant covariates. Estimates obtained from multiple imputation analyses were compared with complete-case analyses as a form of sensitivity analysis. For outcomes or models with complete covariate data, multiple imputation was not applied.

All analyses were 2-sided with a significance threshold of $P < 0.05$. Statistical analyses were performed using SAS Software (Version 9.4, SAS Institute Inc.).

RESULTS

Patient Characteristics

From August 2021 through March 2024, 80 eligible patients were recruited in the trial (median [interquartile range] age, 80 [72–86] years; 44 women [55%]) at 4

Italian academic hospitals equipped with comprehensive stroke centers. 40 patients were randomly assigned to the RIC plus standard medical therapy group and 40 to the control group. Two (5%) patients in the control group and 1 (2.5%) patient in the RIC group were subsequently classified as stroke mimics (Figure 1). Baseline characteristics were balanced between the 2 groups, except for the proportion of large vessel occlusion strokes,

which was higher in the control group than in the RIC group. Baseline patient characteristics are summarized in Table 1. All analyses were performed on the intention-to-treat population (n=80). Missing data were minimal across study variables, with complete follow-up available for all randomized patients and nearly complete covariate data (78/80 patients included in adjusted analyses). Results from multiple imputation analyses were

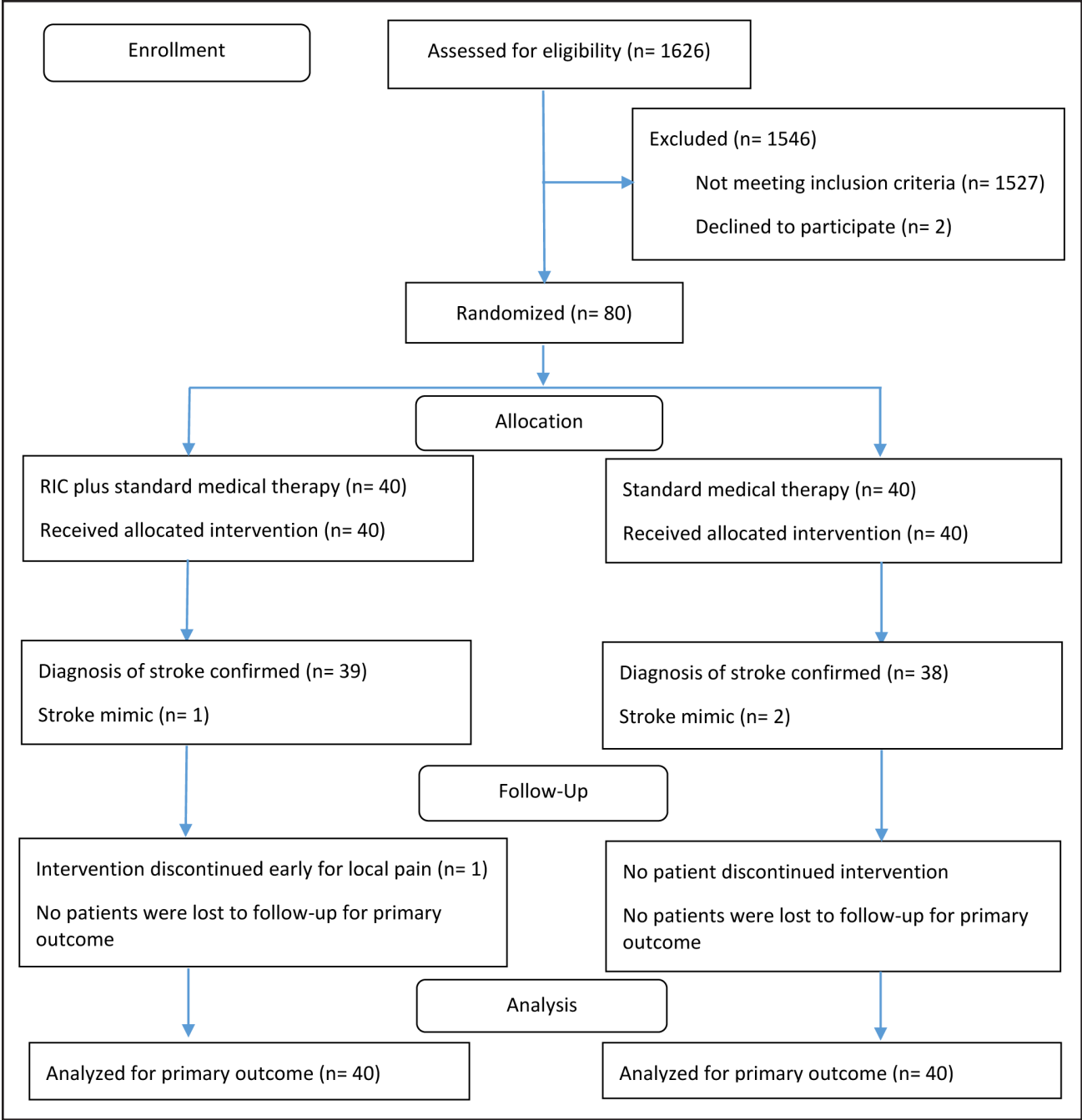


Figure 1. Consolidated Standards of Reporting Trials flow diagram. Of 1626 patients screened for eligibility, 1546 were excluded. Eighty patients were randomized equally to receive remote ischemic conditioning (RIC) plus standard medical therapy (intervention group) or standard medical therapy alone (control group). All patients received the assigned treatment, and no participants were lost to follow-up. All 80 patients were included in the intention-to-treat analysis.

Table 1. Baseline Patient's Characteristics

Variable	RIC group (N=40)	Control group (N=40)
Age, y, median (IQR)	80 (73–86)	82 (71–88)
Male sex, n (%)	22 (55.0)	14 (35.0)
Race and ethnicity, n (%)		
White	39 (97.5)	40 (100)
Asian	...	...
Hispanic	1 (2.5)	...
Black	...	...
Others	...	...
Hypertension, n (%)	29 (74.4)	31 (79.5)
Hyperlipidemia, n (%)	15 (38.5)	19 (48.7)
Obesity, n (%)	5 (12.8)	6 (15.4)
Atrial fibrillation or flutter, n (%)	12 (30.8)	11 (28.2)
Peripheral vascular disease, n (%)	1 (2.6)	3 (7.7)
Coronary artery disease, n (%)	8 (20.5)	9 (23.1)
Chronic heart failure, n (%)	4 (10.3)	8 (20.5)
Valvulopathy, n (%)	5 (12.8)	6 (15.4)
Previous ischemic stroke, n (%)	5 (12.8)	7 (17.9)
Previous TIA, n (%)	2 (5.1)	2 (5.1)
Smoking history, n (%)	12 (30.8)	7 (17.9)
Medications, n (%)		
Antiplatelet therapy	10 (25.6)	14 (35.9)
Oral anticoagulants	13 (33.3)	10 (25.6)
Statins	13 (33.3)	14 (35.9)
Antihypertensive treatment	29 (74.4)	30 (76.9)
Admission NIHSS score		
Median	9	9
Interquartile range	6–13	7–14
Baseline mRS score		
Median	0	0
Interquartile range	0–1	0–1
Time to randomization, h		
Median	6	7
Interquartile range	4–8	5–8
BMI		
Median	25.0	23.9
Interquartile range	23.3–27.2	20.8–25.9
Systolic blood pressure, mm Hg		
Median	153	150
Interquartile range	140–180	138–166
Blood glucose, mg/dL		
Median	133	115
Interquartile range	111–157	105–151
Imaging modality, n (%)		
CT	37 (92.5)	36 (90.0)
MRI	3 (7.5)	4 (10.0)
Large vessel occlusion,* n (%)		
Absent	27 (67.5)	14 (35.0)

(Continued)

Table 1. Continued

Variable	RIC group (N=40)	Control group (N=40)
Present	10 (25.0)	19 (47.5)
Unknown	3 (7.5)	7 (17.5)
Ischemic stroke lesion, n (%)		
No acute lesion	22 (59.5)	21 (58.3)
Early signs of ischemia	7 (18.9)	8 (22.2)
Visible ischemic lesion	8 (21.6)	7 (19.4)
Missing data	3	4
TOAST classification, n (%)		
Large artery atherosclerosis	4 (10.3)	6 (15.4)
Cardioembolism	16 (41.0)	14 (35.9)
Small vessel disease	7 (17.9)	2 (5.1)
Other determined etiologies	0 (.0)	2 (5.1)
Undetermined cause	12 (30.0)	14 (35.0)
Stroke mimics, n (%)	1 (2.5)	2 (5.0)

BMI indicates body mass index; CT, computed tomography; IQR, interquartile range; MRI, magnetic resonance imaging; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; RIC, remote ischemic conditioning; TIA, transient ischemic attack; and TOAST, Trial of ORG 10172 in Acute Stroke Treatment.

*Large vessel occlusions included intracranial internal carotid artery, segment M1 of the middle cerebral artery, A1 segment of the anterior cerebral artery, intracranial vertebral artery, basilar artery, P1 segment of the posterior cerebral artery.

consistent with complete-case analyses across all primary and secondary outcomes, with no meaningful differences in effect estimates or statistical significance (Table S1).

Efficacy Outcomes

Although slightly in favor of RIC treatment, the median percentage change in the NIHSS score at 72 hours was not significantly different between the RIC group and the control group even after adjusting for the covariates of age, sex, cardiovascular risk factors and presence of intracranial vessel occlusion (23.6% versus 29.9%; adjusted coefficient, -3.8 [95% CI, -19.7 to 12.1]; $P=0.635$). Similarly, no significant between-group differences in the median percentage change in the NIHSS score at 24 hours (0.0% versus 0.0%; adjusted coefficient, -7.1 [95% CI, -19.7 to 5.6]; $P=0.267$) nor at 48 hours (18.3% versus 20.5%; adjusted coefficient, -3.9 [95% CI, -19.2 to 11.4]; $P=0.612$) were found. The proportion of patients with poor functional outcome at 90 days did not differ significantly in the 2 groups after adjusting for the abovementioned covariates (57.5% versus 65.0%; adjusted odds ratio, 0.79 [95% CI, 0.21–2.97]; $P=0.732$; Table 2). Post hoc subgroup analyses according to treatment timing (≤ 6 versus >6 hours from symptom onset) did not show a significant difference in the effect of RIC on the primary outcome, with consistent results across time strata and no evidence of a time-dependent treatment effect (Table 3);

Table 2. Efficacy Outcomes

Outcome	RIC group (n=40)	Control group (n=40)	Unadjusted coefficient estimate or OR (CI 95%)	Adjusted coefficient estimate or OR (CI 95%)	P value*
Primary efficacy outcome					
NIHSS score % change at 72 h, median (IQR)	23.6 (0.0 to 50.0)	29.9 (0.0 to 53.6)	−0.9 (−14.6 to 12.9)	−3.8 (−19.7 to 12.1)	0.635
Secondary efficacy outcomes					
NIHSS score % change at 24 h, median (IQR)	0.0 (0.0 to 31.0)	0.0 (0.0 to 35.7)	−3.5 (−14.1 to 7.0)	−7.1 (−19.7 to 5.6)	0.267
NIHSS score % change at 48 h, median (IQR)	18.3 (0.0 to 44.2)	20.5 (0.0 to 50.0)	−1.0 (−14.0 to 12.0)	−3.9 (−19.2 to 11.4)	0.612
mRS score >2 at 90 d, n (%)†	23 (57.5)	26 (65.0)	0.73 (0.30 to 1.8)	0.79 (0.21 to 2.97)	0.732
Primary safety outcome					
Symptomatic intracerebral hemorrhage‡	0 (0.0)	1 (2.5)	...	...	...

IQR indicates interquartile range; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; RIC, remote ischemic conditioning; and SITS-MOST, Safe Implementation of Thrombolysis in Stroke–Monitoring Study.

*The *P* value was calculated using a logistic regression model adjusted for age, sex, cardiovascular risk factors, and presence of intracranial occlusion.

†Poor functional outcome at 90 days was defined as a score >2 (mRS score 3–6) on the mRS, which ranges from 0 to 6, with higher scores indicating more severe disability.

‡According to the SITS-MOST, symptomatic intracerebral hemorrhage was defined as a local or remote type 2 parenchymal hemorrhage with a neurological deterioration of 4 NIHSS score points from baseline or leading to death within 24 hours.

multiple imputation analyses yielded similar results (data not shown).

Safety Outcomes

Symptomatic intracerebral hemorrhage occurred in 0 patients in the RIC group as compared with 1 patient in the control group (Table 2).

Feasibility and Tolerability

39 (97.5%) patients randomized to the RIC group completed RIC in full; only one patient (2.5%) had intervention interrupted due to pain in the treated arm. Wong-Baker Faces Pain Rating Scale score was obtained from all patients who were able to communicate about their pain. 30 patients in the RIC group and 19 patients in the control group were able to complete the scale early after treatment. Among them, 7 (23%) patients in the RIC group scored >0 on the Wong-Baker Scale compared with 0 (0%) patients in the control group, *P*=0.033.

Overall, 32 patients in the RIC group and 33 patients in the control group were able to complete the scale at 72 hours. Among them, 1 (3.1%) patient in the RIC group expressed arm pain or discomfort compared with 2 patients (6.1%) in the control group (odds ratio, 0.50 [95% CI, 0.04–5.80]; *P*=0.580; Table 4).

Exploratory Analysis on RIC Biomarkers

Blood concentrations of circulating biomarkers at 24 and 72 hours in each treatment arm are represented in Figure 2. The median [interquartile range] difference between HIF-1α levels at 72 and 24 hours was 14.9 (−7.4 to 29.4) in the intervention group and −5.2 (−67.8 to 26.5) in the control group. The median difference between HSP27 levels at 72 and 24 hours was −27.8 (−296.3 to 181.3) in the intervention group and 11.7 (−75.3 to 259.8) in the control group. No significant changes in circulating biomarkers were found across the 2 time points in either treatment arm. Furthermore, no significant differences in HIF-1α or HSP27 levels at 72 hours compared with 24 hours were observed between the treatment arms after adjusting for covariates (*P*=0.383 for HSP27; *P*=0.257 for HIF-1α; Table 5).

DISCUSSION

This trial was designed to evaluate the neuroprotective effect of RIC in patients with moderate-to-severe acute ischemic stroke who are ineligible for recanalization therapies. Unlike most previous trials^{9,18–22} that enrolled patients eligible to intravenous thrombolysis or mechanical thrombectomy, TRICS-9 focused on a population without access to recanalization therapies to minimize

Table 3. Post Hoc Analysis of the Primary Outcome According to Treatment Time Window

Time window	% change NIHSS score at 72 h	RIC group	Control group	P value*	Adjusted estimate† (95% CI)	P value
≤6 h	Median (IQR)	33.3 (16.7 to 50.0)	44.4 (6.7 to 70.0)	0.4675	1.0 (−33.4 to 35.5)	0.9499
>6 h	Median (IQR)	16.7 (0.0 to 28.6)	25.0 (0.0 to 50.0)	0.7291	−4.3 (−22.5 to 13.8)	0.6339

IQR indicates interquartile range; NIHSS, National Institutes of Health Stroke Scale; and RIC, remote ischemic conditioning.

**P* values from Wilcoxon rank-sum test.

†Adjusted estimates derived from generalized linear models (GLM) including age, sex, cardiovascular risk factors, and presence of intracranial occlusion.

Table 4. Tolerability of RIC

Outcome	RIC group*	Control group*	OR (CI 95%)	P value
Wong-Baker Faces Pain Rating Scale† score >0 after treatment, n/tot (%)	7/30 (23.3)	0/19 (.0)	...	0.033‡
Wong-Baker Faces Pain Rating Scale score >0 at 72 h, n/tot (%)	1/32 (3.1)	2/33 (6.1)	0.50 (0.04–5.80)	0.580‡

n/tot indicates number out of total; OR, odds ratio; and RIC, remote ischemic conditioning.

*30 patients in the RIC group and 19 patients in the control group were able to complete the scale early after treatment. 32 patients in the RIC group and 33 patients in the control group were able to complete the scale at 72 hours.

†Wong-Baker Faces Pain Rating Scale scores pain from 0 to 10, with 0 indicating no pain and 10 worst pain.

‡The P value was calculated using the χ^2 test.

§The P value was calculated using a logistic regression model.

confounding from highly effective treatments and enable a more direct assessment of the biological and clinical effects of RIC.

The 9-hour therapeutic window for the application of RIC was predefined in the study protocol²⁵ and was inspired by trials showing benefit of recanalization therapies in extended therapeutic windows in the presence of salvageable brain tissue, as well as by preclinical studies showing that RIC promotes penumbral survival and remains effective several hours after stroke onset.^{27,28}

The present trial did not show evidence of benefit from RIC on early neurological improvement or 90-day disability. These neutral findings align with results from several prior randomized trials^{18–21} suggesting that the clinical efficacy of RIC in acute ischemic stroke is still unestablished.

The primary end point—percent change in NIHSS score at 72 hours—was selected based on methodological advantages demonstrated in the reanalysis of the National Institute of Neurological Disorders and Stroke Part 1 trial.²⁶ This continuous metric accounts for heterogeneity in baseline stroke severity, increases sensitivity to detect treatment-related changes, and provides more statistical power than absolute NIHSS score changes or dichotomized outcomes, especially in trials with modest sample sizes. Early neurological improvement was intended to capture a proximal signal of biological activity, less influenced by postacute care variability and rehabilitation intensity than longer-term disability end points. A 20% between-group difference was considered clinically meaningful from power calculations planned based on expected dispersion at 72 hours in the prespecified statistical analysis.²⁵ Although 72-hour improvement is a short-term outcome, early neurological changes have been shown to correlate with long-term functional outcome^{29,30} and were complemented in our study by assessment of 90-day disability. Since early neurological fluctuations are common in acute ischemic stroke, serial NIHSS score assessments at 24, 48, and 72 hours were

used in this trial, which showed consistent trajectories across time points, without differential patterns between treatment groups. Moreover, neurological worsening was conservatively handled according to the prespecified statistical plan, thereby limiting the risk of overestimating treatment-related improvement.

RIC was applied by 4 cycles of 5-minute upper limb ischemia followed by reperfusion. Although this single-session approach reflects the design of earlier studies available at the time of protocol development,^{8,9,18–21} it may have provided insufficient stimulus to induce a sustained biological effect. Overall, the neutral results across both early and late outcomes suggest that this RIC regimen did not confer clinical benefit in this population. While preclinical studies have demonstrated that repeated RIC sessions produce significant neuroprotection,^{1–35,6,13–16} clinical evidence has been variable. For instance, the RECAST 2 trial (Remote ischaemic Conditioning After Stroke Trial 2)⁹ found no benefit with consecutive daily sessions, whereas the RICAMIS²³ trial reported improved outcomes with a 2-week regimen, and the REMOTE-CAT²¹ trial showed modest trends toward improved disability at 3 months with a 2-session protocol. These findings indicate that frequency, duration, and intensity of conditioning may be critical determinants of efficacy. Accordingly, heterogeneity in conditioning paradigms across trials represents a key source of variability and should be considered when interpreting and comparing clinical results.

Multiple preclinical studies showed that RIC can exert neuroprotection even in the case of persistent artery occlusion. The parallel TRICS BASIC preclinical trial (Multicenter Translational Trial of Remote Ischemic Conditioning in Acute Ischemic Stroke),³¹ conducted with harmonized methodology across 2 rodent species, showed modest but significant improvements in functional and histological outcomes. Similarly, in multiple rodent models of middle cerebral artery occlusion, RIC reduced infarct volume and improved neurological outcomes despite ongoing large vessel occlusion.^{13–17} These findings indicate that vessel recanalization is not a prerequisite for RIC efficacy. Importantly, even in the absence of reperfusion therapy, residual perfusion through leptomeningeal collateral circulation provides biological access to the ischemic penumbra. Indeed, collateral status is a major determinant of tissue survival and clinical outcome in acute ischemic stroke,^{32,33} and experimental evidence suggests that RIC itself may enhance collateral flow.⁷ Thus, circulating protective mediators induced by RIC may reach at-risk tissue via preserved microvascular pathways. Moreover, experimental studies implicate activation of HSP27 pathways,^{1–3} HIF-1 α signaling with modulation of inflammatory responses,⁵ nitric oxide-dependent mechanisms,⁶ neuroglobin upregulation,¹⁷ and nNOS (neuronal NO synthase)/ERK (extracellular signal-regulated kinase) signaling cascades,¹⁶ suggesting that RIC-mediated protection appears to be

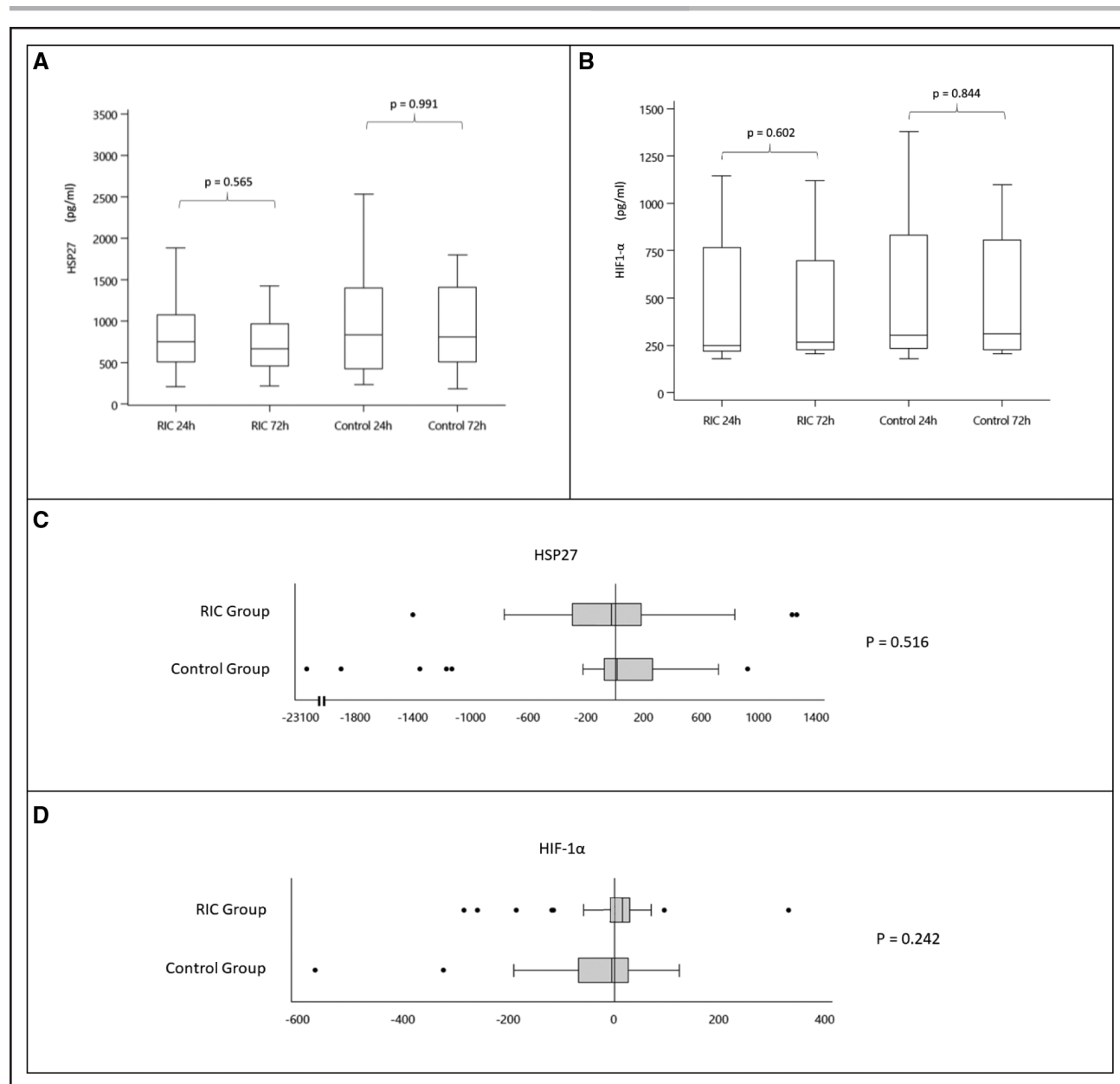


Figure 2. Blood concentrations of circulating biomarkers at 24 and 72 hours in each treatment arm.

Box plots show plasma concentrations of HSP27 (heat shock protein 27; **A**) and HIF-1 α (hypoxia-inducible factor 1-alpha; **B**) at 24 and 72 hours after enrollment in patients receiving remote ischemic conditioning (RIC) plus standard medical therapy (RIC group) and in those receiving standard medical therapy alone (control group). No significant within-group differences were observed across time points for either biomarker (P values were calculated using the Wilcoxon test). Box plots also show the 24- and 72-hour changes in plasma HSP27 (**C**) and HIF-1 α (**D**) in each treatment group. No significant differences in biomarker changes were detected between treatment arms. P values were calculated using the Wilcoxon test.

multimodal rather than exclusively dependent on circulating mediators.

The contrast between preclinical observations and the neutral clinical findings from the present study highlights the longstanding translational gap in neuroprotection research and emphasizes the need to refine experimental alignment and dosing paradigms when advancing preclinical therapies to clinical testing.

RIC was well tolerated, with only one discontinuation due to local discomfort and a low overall burden of

transient arm pain, confirming its excellent feasibility and safety profile. Importantly, the risk of symptomatic intracerebral hemorrhage did not differ between treatment arms, further supporting the safety of the intervention.

Unlike most previous trials that primarily focused on clinical outcomes, our study also examined 2 biomarkers—HIF-1 α and HSP27—widely investigated in preclinical models of RIC-induced neuroprotection. This dual aim reflects a translational approach aimed at bridging mechanistic findings with clinical application. Despite

Table 5. Exploratory Analysis on Circulating Levels of HIF-1α and HSP27

RIC biomarker	RIC group (n=35)	Control group (n=36)	Unadjusted estimate (CI 95%)	Adjusted estimate* (CI 95%)	P value†
HIF-1α Diff72,‡ median (IQR)	14.9 (−7.4 to 29.4)	−5.2 (−67.8 to 26.5)	33.0 (−21.0 to 87.0)	34.9 (−26.2 to 96.1)	0.257
HSP27 Diff72,§ median (IQR)	−27.8 (−296.3 to 181.3)	11.7 (−75.3 to 259.8)	558.5 (−808.0 to 1925.0)	663.0 (−849.2 to 2175.1)	0.383

Multiple imputation was not required, as no missing covariate data were present for these analyses. Diff72 indicates difference at 72 hours compared to 24 hours; HIF-1α, s hypoxia-inducible factor 1-alpha; HSP27, heat shock protein 27; IQR, interquartile range; and RIC, remote ischemic conditioning.

*The P value was calculated using a generalized linear model adjusted for age, sex, cardiovascular risk factors, and presence of intracranial occlusion.

†Adjusted models include age, sex, and vascular risk factors.

‡HIF-1α Diff72 indicates the difference between plasma concentration of HIF-1α at 24 and 72 hours.

§HSP27 Diff72 indicates the difference between plasma concentration of HSP27 at 24 and 72 hours.

their biological relevance, no treatment-related differences were detected at 24 or 72 hours. These findings are consistent with a prior clinical study⁹ and suggest that peripheral biomarker responses may be heterogeneous, time-dependent, or inadequately captured by sampling at limited sampling points. Sampling was aligned with clinical end points to ensure coherence between biological and clinical trajectories; however, early transient changes occurring before 24 hours cannot be excluded. Given the exploratory nature of these analyses, the study was not powered to detect between-group differences, and findings should be interpreted with caution.

A distinctive feature of our cohort is the advanced age of the enrolled patients, which exceeds that reported in most previous trials.^{8,21,23} Advanced age may plausibly influence responsiveness to RIC. Experimental data show that aging attenuates endogenous protective pathways involved in ischemic conditioning, including stress-response signaling and mitochondrial function, resulting in reduced cardioprotection in aged animals.^{34,35} In the stroke field, a recent age-stratified analysis of RIC in acute ischemic stroke suggested that treatment efficacy may decline with advancing age, particularly in patients ≥80 years, although without a statistically significant interaction.³⁶ Therefore, our neutral findings may partly reflect reduced biological responsiveness in very old patients, and caution is warranted when extrapolating results to younger stroke populations.

To conclude, a single session of RIC applied within 9 hours of symptom onset in patients with acute ischemic stroke ineligible for recanalization therapies did not demonstrate clinical benefit under the conditions of the present trial. Future trials may require more intensive or prolonged conditioning protocols, biomarker-guided patient stratification, and closer alignment of biological sampling windows with mechanistic data from animal models to accurately assess the therapeutic potential of RIC in this setting.

This study has several limitations. First, the modest sample size may have limited the ability to detect small-to-moderate treatment effects. The trial was powered to detect a relatively large predefined difference in early neurological improvement; therefore, smaller effect sizes cannot be excluded and may have been missed. Second,

the single-session RIC protocol may have been insufficient to trigger clinically relevant neuroprotective cascades, as suggested by studies employing repeated or extended conditioning regimens. Third, inclusion of both anterior and posterior circulation strokes—although necessary to ensure feasibility—introduced pathophysiological heterogeneity. However, given the small proportion of posterior circulation strokes, their inclusion in the primary intention-to-treat analysis is unlikely to have influenced the results. Recruitment challenges required protocol amendments and extended enrollment, and further expansion would have jeopardized feasibility. Nevertheless, the trial maintained rigorous multicenter randomization and excellent adherence to the intervention. Fourth, biomarker sampling was limited to 2 time points, potentially missing early or transient molecular changes and reducing the ability to detect subtle peripheral signatures of central neuroprotection. Fifth, collateral status and baseline infarct size were not systematically collected and could not be included in adjusted analyses, limiting adjustment for key prognostic imaging variables and potentially introducing residual confounding. Sixth, patients with neurological deterioration were assigned a 0% change, a conservative approach aimed at limiting the influence of negative outliers in a modest sample, though this strategy may have attenuated detectable between-group differences. Finally, the open-label design, although mitigated by blinded outcome assessment, may carry residual risk of performance bias.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Table S1

CONSORT Checklist

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