

## Original Research

# Efficacy of remote limb ischemic conditioning on poststroke cognitive impairment

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The impact of remote limb ischemic conditioning on post-stroke cognitive impairment was evaluated with 104 first-time patients of noncardiac ischemic stroke. During the acute phase the patients were randomized into control and remote limb ischemic conditioning groups. Both groups received standard treatment, while the remote limb ischemic conditioning group received additional remote limb ischemic conditioning treatment for 6 months. All participants underwent neuropsychological evaluation, transcranial Doppler detection, P300 event-related potential and brachial-ankle pulse wave velocity measurements, and determination of serum intercellular adhesion molecule-1 and endothelin-1 levels both at admission and 6 months poststroke. The number of cases with post-stroke cognitive impairment in each group was evaluated 6 months poststroke. No statistically significant difference was found in demographic data or baseline detection indices at admission between the two groups. However, at 6 months poststroke, the remote limb ischemic conditioning group had significantly higher total Montreal Cognitive Assessment score and its domains of visuospatial and executive functioning and attention scores, significantly lower activity of daily living scale score, shorter P300 latency, and higher amplitude compared with the control group. Moreover, the middle cerebral artery, average blood flow velocity was significantly higher, while the middle cerebral artery-pulsation index, basilar artery pulsation index, and the levels of brachial-ankle pulse wave velocity, intercellular adhesion molecule-1, and endothelin-1 were significantly lower in the remote limb ischemic conditioning group compared with the control group. These results demonstrate that remote limb ischemic conditioning causes a significant improvement in cognitive domains, such as visuospatial and executive functioning and attention, and is therefore linked with reduced incidence of poststroke cognitive impairment.

## Keywords

Event-related potential P300; stroke; cognitive impairment; ischemic conditioning; remote limb; vascular neurology

## 1. Introduction

A commonly occurring complication of stroke is the post-stroke cognitive impairment (PSCI) refers to a range of syndromes that meet the diagnostic criteria for cognitive disorders within 6 months after the clinical event of a stroke. These vary from conditions like "poststroke cognitive impairment no dementia" (PSCIND) to "poststroke dementia" (PSD), which not only enhances patient mortality but also severely impairs their normal daily life and social ability. Prevention and early intervention are, therefore crucial for alleviating PSCI.

Distant ischemic adaptation or remote ischemic conditioning (RIC) is a new method for the prevention and treatment of cerebrovascular disease. In principle, RIC applies transient ischemic stimulation to one organ to induce an endogenous protective response against severe ischemic injury in another distant organ. For example, remote limb ischemic conditioning (RLIC) applies intermittent interruptions of blood supply to non-vital body organs (the limb) to protect vital organs (the heart or brain) from ischemic injury (Brandli, 2015; Pan et al., 2016).

In general, RIC is divided into distinct types based on the time sequence of stimulation and organ injury: distant or remote ischemic preconditioning (RIPreC), distant ischemia-reperfusion adaptation or RIPerC, and distant ischemia adaptation of remote ischemic postconditioning (RIPostC) (Hess et al., 2015) refers to the protective response induced by transient ischemic stimulation of a distant organ after ischemia-reperfusion injury.

Multiple studies have indicated that RLIC reduces the risk of cerebral infarct and intracranial atherosclerotic stenosis, and decelerates cognitive deterioration in patients with cerebral small-vessel disease (Gonzalez et al., 2014; Hougaard et al., 2013; Meng et al., 2012, 2015; Nikkola et al., 2015; Wang et al., 2017a). However, the impact of RLIC on PSCI has not been clearly defined. The present study analyzes the impact of RLIC performed during the acute phase of ischemic stroke on PSCI to elaborate its mechanism of action. For the evaluation of cognitive function, we used the Montreal Cognitive Assessment (MoCA) scale for neuropsychological evaluation and P300 for objective evaluation. Previous studies show that P300 can be used to identify the early stages of dementia and to distinguish between dementia and depression-related pseudodementia. The efficacy of drugs to improve cogni-

Table 1. Comparison of baseline data between control and RLIC group patients

Variable	Control group (n = 44)	RLIC group (n = 42)	$\chi^2$ value or t value	P value
Age (year, mean $\pm$ s)	63.91 $\pm$ 7.61	64.16 $\pm$ 7.71	-0.15	0.881
Sex (male vs female)	26 : 18	28 : 14	0.528	0.468
BMI (kg/m <sup>2</sup> ) (mean $\pm$ s)	24.20 $\pm$ 2.77	24.29 $\pm$ 3.05	-0.627	0.532
Hypertension (number of patients, %)	24 (54.54)	20 (47.62)	0.174	0.688
Diabetes (number of patients, %)	14 (31.82)	14 (33.33)	0.095	0.757
Coronary heart diseases (number of patients, %)	12 (28.57)	13 (30.95)	0.056	0.813
Smoking (number of patients, %)	20 (45.45)	22 (52.38)	0.413	0.521
Drinking (number of patients, %)	12 (27.17)	10 (23.81)	0.135	0.713
Duration of education (year, mean $\pm$ s)	8.80 $\pm$ 3.81	8.50 $\pm$ 3.78	0.361	0.719
LDL-C (mmol/L, mean $\pm$ s)	3.80 $\pm$ 0.64	3.72 $\pm$ 0.74	0.527	0.6
Homocysteine (mmol/L, mean $\pm$ s)	17.23 $\pm$ 2.66	17.68 $\pm$ 3.13	-0.708	0.481
Uric acid (mmol/L, mean $\pm$ s)	366.16 $\pm$ 69.88	356.69 $\pm$ 60.38	0.671	0.504
Site of cerebral infarct				
Anterior vs posterior circulation	30 : 14	27 : 15	0.146	0.702
Anterior circulation (left vs right)	16 : 14	14 : 13	0.153	0.696
Cerebral infarct volume (cm <sup>3</sup> , mean $\pm$ s)	4.69 $\pm$ 0.32	4.61 $\pm$ 0.39	1.001	0.089
Time of stroke onset (day, mean $\pm$ s)	1.86 $\pm$ 1.08	1.95 $\pm$ 1.09	-0.38	0.705
NIHSS score (point, mean $\pm$ s)	4.07 $\pm$ 1.69	4.19 $\pm$ 1.49	-0.356	0.723

tive function can also be reliably evaluated by P300, which is an effective tool for clinical evaluation of cognitive function (Fabrizio and Sara, 2011; Parra et al., 2012).

## 2. Materials and methods

### 2.1 Participants

One hundred four patients with first-time noncardiac ischemic stroke during the acute phase were enrolled at the Department of Neurology, Zhabei Central Hospital, Jing'an District, Shanghai, People's Republic of China, between July 2018 and March 2019. All patients meeting the inclusion criteria (see Section 2.2) were randomized (using a random number table) into either control (n = 52) or RLIC (n = 52) group. Both groups received the standard treatment, while the RLIC group received additional RLIC treatment for 6 months. The protocol for this study was approved by the ethics committee of Zhabei central hospital (Approval#: ZBLL2018062605). All patients or their relatives provided signed informed consent for their participation.

By the end of the study, 8 patients were withdrawn or excluded from the control group (4 cases lost to six-months follow up, 4 cases received other treatments), 10 patients from RLIC group (5 cases discontinued intervention, 3 cases lost to six-months follow up, 2 cases received other treatments). Thus, a total of 44 patients in the control group and 42 patients in RLIC group completed the study, leading to 86 patients enrolled in the study overall. Comprehensive demographic data were collected for all patients, including sex, age, body mass index (BMI), National Institute of Health Stroke Scale (NIHSS) score; history of hypertension, smoking, diabetes, and drinking. Data were comparable for all categories between the two groups and showed no statistically significant differences ( $P > 0.05$ ) (Table 1).

### 2.2 Criteria for inclusion, exclusion, elimination, and withdrawal

The inclusion criteria for the study were defined as follows: (1) symptoms of focal neurological deficit and new cerebral infarct confirmed by magnetic resonance diffusion-weighted imaging to meet the diagnostic criteria for cerebral infarct, ( $> 4.5$  h after disease onset, beyond the indications of thrombolytic therapy); (2) age between 18 and 80 years; (3) stabilized vital signs and neurological symptoms for 24 h; and (4)  $\leq 14$  days after disease onset.

The exclusion criteria were defined as follows: (1) patients with cognitive impairment prior to the stroke, as indicated by the informant questionnaire on cognitive decline in the elderly; (2) patients with disorders of consciousness; (3) patients with neurological diseases, such as brain trauma, epilepsy, encephalitis, and normal intracranial pressure hydrocephalus, which may cause cognitive disorders; (4) other brain diseases, such as brain tumors or multiple sclerosis, confirmed by computerized tomography (CT)/magnetic resonance imaging (MRI); (5) patients who scored  $> 7$  points on Hamilton depression (HAMD) scale or who present other mental illnesses; (6) apparent aphasia and/or dysarthria affecting cognitive function assessment; (7) intracranial/external artery stenosis  $> 50\%$  (degree of intracranial artery stenosis analyzed by transcranial Doppler (TCD): mean flow velocity  $> 90$  cm/s for intracranial internal carotid artery, 100 cm/s for MCA,  $> 80$  cm/s for basilar artery or vertebral artery measured according to the stroke outcomes and neuroimaging of intracranial atherosclerosis criteria (Deweese et al., 1970; Zhao et al., 2011); degree of extracranial artery stenosis evaluated by carotid duplex ultrasound); (8) systolic blood pressure recording  $> 200$  mm Hg; (9) use of psychotropic drugs, antidepressants, and/or drug abuse or addiction (such as sedatives, hypnotics, narcotic medication, etc.) which can affect cognitive functions and cognitive assessment.

Table 2. Comparisons of MoCA and ADL scores between control and RLIC group patients at admission and at 6 months poststroke (point; mean  $\pm$  s)

Item	At admission				At 6 months poststroke			
	Control group	RLIC group	<i>t</i> value	<i>P</i> value	Control group	RLIC group	<i>t</i> value	<i>P</i> value
MoCA	25.16 $\pm$ 2.61	24.86 $\pm$ 2.55	0.543	0.589	23.82 $\pm$ 2.61	25.02 $\pm$ 1.97	-2.411	0.018
ADL	23.91 $\pm$ 3.16	24.98 $\pm$ 4.26	-1.324	0.189	23.80 $\pm$ 2.28	20.86 $\pm$ 4.17	2.603	0.011

Table 3. Comparison of each MoCA domain score between control and RLIC group patients at admission and 6 months poststroke (point; mean  $\pm$  s)

Item	At admission				At 6 months poststroke			
	Control group	RLIC group	<i>t</i> value	<i>P</i> value	Control group	RLIC group	<i>t</i> value	<i>P</i> value
Visuospatial and executive functioning	4.35 $\pm$ 0.97	4.28 $\pm$ 0.67	1.441	0.749	3.63 $\pm$ 1.01	4.23 $\pm$ 0.73	-2.201	0.023
Naming	2.21 $\pm$ 0.70	2.17 $\pm$ 0.50	1.042	0.803	2.19 $\pm$ 0.78	2.17 $\pm$ 0.40	0.87	0.589
Attention	5.27 $\pm$ 0.54	5.20 $\pm$ 0.93	0.772	0.67	4.62 $\pm$ 0.41	5.31 $\pm$ 0.91	-1.901	0.031
Language	2.30 $\pm$ 0.39	2.27 $\pm$ 0.35	0.782	0.595	2.35 $\pm$ 0.56	2.32 $\pm$ 0.57	0.677	0.809
Abstract	1.61 $\pm$ 0.27	1.60 $\pm$ 0.23	0.353	0.445	1.59 $\pm$ 0.72	1.60 $\pm$ 0.33	0.634	0.845
Delayed recall	4.38 $\pm$ 0.35	4.25 $\pm$ 0.56	0.428	0.573	4.41 $\pm$ 0.39	4.42 $\pm$ 0.65	-0.301	0.94
Orientation	5.04 $\pm$ 0.28	5.09 $\pm$ 1.01	-0.509	0.665	5.03 $\pm$ 0.87	4.97 $\pm$ 1.09	0.234	0.378

Table 4. Comparison of P300 event-related potential latency (ms) and amplitude ( $\mu$ V) between control and RLIC group patients at admission and 6 months poststroke

Item	At admission				At 6 months poststroke			
	Control group	RLIC group	<i>t</i> value	<i>P</i> value	Control group	RLIC group	<i>t</i> value	<i>P</i> value
P300 latency (ms, mean $\pm$ s)	369.34 $\pm$ 39.26	375.47 $\pm$ 45.93	-0.723	0.726	373.56 $\pm$ 64.72	331.70 $\pm$ 46.28	0.174	0.032
P300 amplitude (mV, mean $\pm$ s)	6.87 $\pm$ 3.94	7.04 $\pm$ 5.45	-0.557	0.835	6.75 $\pm$ 1.98	8.52 $\pm$ 5.27	-0.152	0.027

Table 5. Comparison of blood flow indices, baPWV, and levels of ICAM-1 and ET-1 between control and RLIC group patients at admission and 6 months poststroke

Item	At admission				At 6 months poststroke			
	Control group	RLIC group	<i>t</i> value	<i>P</i> value	Control group	RLIC group	<i>t</i> value	<i>P</i> value
TCD								
MCA-Vm (cm/s)	72.37 $\pm$ 19.13	70.80 $\pm$ 17.25	0.424	0.482	66.56 $\pm$ 10.63	80.39 $\pm$ 9.94	-1.159	0.035
MCA-PI	1.19 $\pm$ 1.13	1.17 $\pm$ 0.65	0.562	0.745	1.17 $\pm$ 0.90	1.02 $\pm$ 0.67	1.327	0.019
ACA-Vm (cm/s)	53.56 $\pm$ 10.81	55.34 $\pm$ 9.01	-0.433	0.621	54.01 $\pm$ 8.40	57.45 $\pm$ 9.54	-0.421	0.763
ACA-PI	1.15 $\pm$ 0.93	1.13 $\pm$ 0.75	0.372	0.445	1.14 $\pm$ 0.89	1.11 $\pm$ 0.76	0.572	0.865
BA-Vm (cm/s)	45.73 $\pm$ 7.43	47.25 $\pm$ 10.27	-0.254	0.649	44.34 $\pm$ 7.45	46.91 $\pm$ 7.72	0.563	0.591
BA-PI	1.10 $\pm$ 0.49	1.08 $\pm$ 0.53	0.541	0.772	1.11 $\pm$ 0.75	0.97 $\pm$ 0.68	1.02	0.021
baPWV (m/s)	18.54 $\pm$ 4.55	18.31 $\pm$ 5.03	1.025	0.931	17.51 $\pm$ 5.03	15.35 $\pm$ 4.70	1.125	0.033
ICAM-1 (ng/L)	323.50 $\pm$ 34.12	327.49 $\pm$ 42.30	-1.403	0.765	259.60 $\pm$ 28.38	174.60 $\pm$ 20.02	1.52	0.017
ET-1 (ng/L)	81.42 $\pm$ 12.47	77.96 $\pm$ 14.01	0.943	0.655	50.17 $\pm$ 7.83	36.04 $\pm$ 4.91	1.631	0.032

The elimination and withdrawal criteria were as follows: (1) presence of active intracranial bleeding, pulmonary edema, intracranial infection, and other acute complications of the heart, brain, and kidney; (2) patients who received other treatment(s) during the study; (3) patients who could not complete the study as a result of death or loss to follow-up.

## 2.3 Methods

### 2.3.1 Remote limb ischemic conditioning

The RLIC procedure consisted of 5 cycles (of 5 minutes each) of ischemia applied daily to the upper limb of the side unaffected by the stroke. Ischemia was induced by a noninvasive cuff inflator (from a sphygmomanometer) placed at the site of brachial artery pulsation and inflated to 200 mm Hg, with intervening 5-minute intervals of reperfusion during which the cuff was deflated (Koch et al., 2011). Training and education were provided by the study staff to patients from the RLIC group. Monitoring and instructing follow-ups were performed every 2 weeks for 6 months by telephone after patient discharge.

### 2.3.2 Neuropsychological evaluation/scale

At admission and 6 months poststroke, neuropsychological evaluations were conducted for all patients by at least 2 personnel who had completed standardized training.

(1) Montreal Cognitive Assessment scale: The 2006 Beijing version of MoCA was adopted (Yu et al., 2012). The cognitive domains included visuospatial and executive functioning, naming, attention, language, abstraction, delayed recall, and orientation, with a total score of 30 points. One point was added to the patient's total score if his/her duration of education was  $\leq 12$  years, and a score of 26 and higher were considered normal. While a score of less than 26 was considered PSCI.

(2) Hamilton Depression Scale: The 24-item version was adopted in this study, with higher scores indicating a higher degree of depression. A score  $> 35$  was taken to indicate severe depression; scores between 20-35 suggested mild or moderate depression, while values 8-20 indicated possible depression, and  $< 8$  were considered normal.

(3) The Activity of Daily Living Scale: This evaluation consisted of two domains: physical and instrumental. Physical activity of daily living (ADL) included 6 items (toileting, eating, dressing, grooming/personal hygiene, ambulating, and bathing). Instrumental ADL included 8 items (ability to use a telephone, shopping, food preparation, housekeeping, laundry, mode of transportation, responsibility for own medications, and ability to handle finances). Each item accounted for 4 points, with a total possible score of 56 points. Scores  $< 16$  points were considered normal, while those  $> 16$  points were taken to indicate varying degrees of functional decline.

### 2.3.3 Measurement of P300 event-related potential

All patients received P300 measurement both at admission and 6 months post-stroke, using the electromyography-evoked potential meter (Keypoint, Dantes). In accordance with the International 10-20 system, the electrodes were placed at points Cz and Pz, the grounding wire placed at the midpoint of the forehead, and the reference electrode was placed at the mastoid. An oddball auditory sequence was adopted as the stimulation frequency, with a nontar-

get stimulation frequency of 1 kHz, a target stimulation frequency of 2 kHz, a probability for target stimulation frequency of 20%, a stimulus intensity of 110 dB, and an average superposition of 30 times. P300 waveform recorded at point Cz was considered as the basic waveform, while P300 latency (ms) and amplitude ( $\mu V$ ) were recorded.

### 2.3.4 Transcranial Doppler detection

All patients underwent TCD detection, both at admission and 6 months poststroke, on the Digital TCD System (Digi-Lite, RIMED). The blood flow parameters of the main intracranial arteries were detected through the sacral window and the occipital window, and the mean blood flow velocity (Vm) and vascular pulsation index (PI) were recorded. The middle cerebral artery-PI (MCA-PI), middle cerebral artery mean blood flow velocity (MCA-Vm), anterior cerebral artery pulsation index (ACA-PI), anterior cerebral artery mean blood flow velocity (ACA-Vm), basilar artery mean blood flow velocity (BA-Vm), and basilar artery pulsation index (BA-PI) were compared between the two groups.

### 2.3.5 Brachial-ankle pulse wave velocity

All patients underwent the measurement of brachial-ankle pulse wave velocity (baPWV) both at admission and 6 months poststroke. The VP-1000 Vascular Profiler (BP-203RP III, Omron) was used for these measurements. The patient lay supine with cuffs placed on bilateral elbows and bilateral ankles, while electrocardiogram electrodes and sensors were placed on the wrists and in the precordial area. baPWV measurements were then automatically performed, analyzed, and recorded by the equipment. Measurements were made simultaneously on both sides, with the higher value read as the final baPWV result of the patient.

### 2.3.6 Determination of serum intercellular adhesion molecule-1 and endothelin-1 levels

For all participants, serum samples were isolated from venous blood at admission and 6 months poststroke to measure the levels of intercellular adhesion molecule-1 (ICAM-1) and endothelin-1 (ET-1), using the enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer's instructions.

## 2.4 Statistical analyses

SPSS 23.0 was used for statistical analysis of data. All measurements are expressed as mean  $\pm$  standard deviation. Measurement data were compared between the two groups and analyzed for statistical significance using non-paired and equal variance student's *t*-test. A comparison of count data was performed using the chi-square test. Pearson correlation analysis was used to analyze continuous variables. *P*-value  $< 0.05$  was considered statistically significant.

## 3. Results

### 3.1 Comparison of MoCA and ADL scores between the two groups

As expected from the randomized distribution of patients in the control or RLIC group, the comparison of average MoCA and ADL scores between patients in the two groups revealed no statistically significant difference at admission. However, at 6 months poststroke, patients in the RLIC group had significantly higher

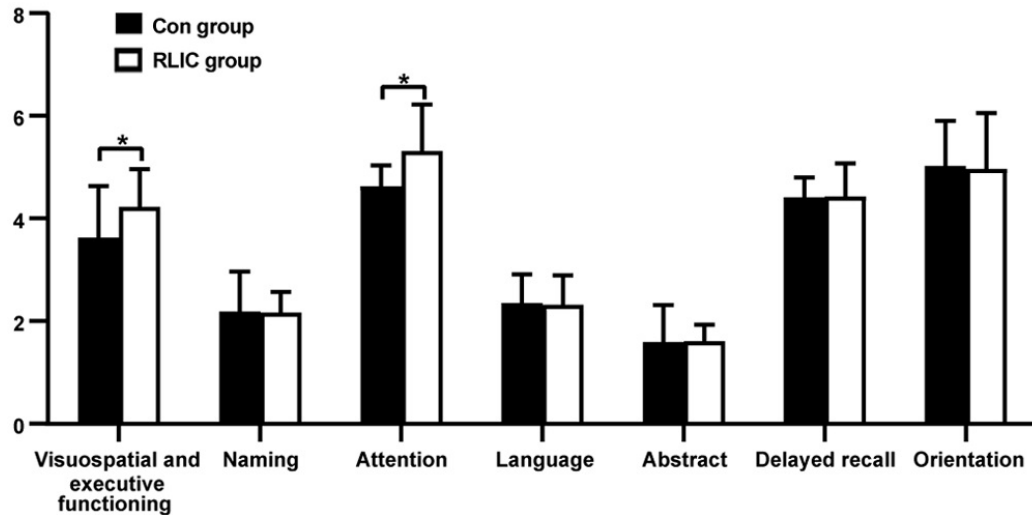


Figure 1. Each MoCA domain score at 6 months poststroke (\*indicates  $P < 0.05$ ): Scores of visuospatial and executive functioning were significantly higher in the RLIC group compared with the Con group ( $P = 0.023$ ), Scores of attention were significantly higher in the RLIC group compared with the Con group ( $P = 0.031$ ).

MoCA scores and lower ADL scores, as compared to the control group ( $P < 0.05$ ) (Table 2). Thus, RLIC group patients reported significantly improved scores in neuropsychological evaluations than control group patients.

Further analysis of each MoCA domain score at 6-months poststroke revealed significantly higher average scores of RLIC patients, specifically in the domains of "visuospatial and executive functioning" and "attention" as compared with the control group (Table 3; Fig. 1).

### 3.2 Comparison of PSCI incidence

To further assess the impact of RLIC treatment, we determined the rate of incidence of PSCI in patients in the two groups. Remarkably, the number of patients with PSCI was found to be significantly lower in the RLIC group (15 patients) than in the control group (28 patients) ( $\chi^2 = 6.701$ ,  $P = 0.010$ ).

### 3.3 Comparison of P300 latency (ms) and amplitude ( $\mu V$ )

We also determined P300 latency and amplitude measurements as an indicator of cognitive function. As expected, P300 latency and amplitude measurements performed at admission detected no statistically significant difference between patients in the control group versus those in RLIC (Table 4). However, at 6 months poststroke, RLIC group patients displayed (on average) shorter P300 latency and higher amplitude than those in the control group, in a manner that was statistically significant on both counts ( $P < 0.05$ ) (Table 4, Fig. 2).

### 3.4 Comparison of blood flow indices, baPWV values, and levels of ICAM-1 and ET-1

At admission, TCD detection showed no significant difference in MCA-Vm, MCA-PI, ACA-Vm, ACA-PI, ACA-Vm, BA-Vm, or BA-PI between patients in the two groups. In contrast, at 6 months poststroke, the average MCA-Vm measurement was significantly higher in the RLIC group than in the control group, while both MCA-PI and BA-PI measurements were significantly lower in the RLIC group than in the control group.

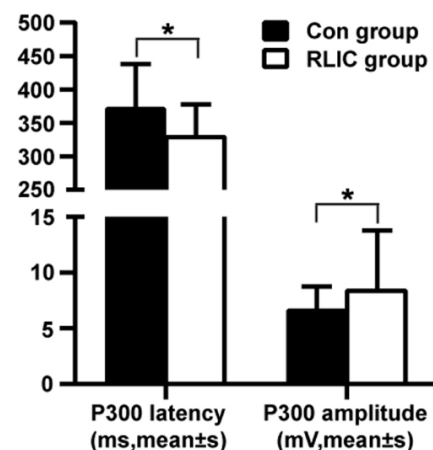


Figure 2. P300 latency (ms) and amplitude ( $\mu V$ ) at 6 months poststroke (\* indicates  $P < 0.05$ ): The P300 latency was shorter in the RLIC group than in the Con group ( $P = 0.032$ ), The amplitude was higher in the RLIC group than in the Con group ( $P = 0.027$ ).

Similarly, no significant difference was found in the baPWV value between the two groups at admission, while the baPWV value was lower in the RLIC group than in the control group at 6 months poststroke. Finally, while ICAM-1 and ET-1 protein levels were comparable in the two groups of patients at admission, both ICAM-1 and ET-1 levels were found to be lower in the RLIC group (compared to control) at 6 months poststroke (Table 5; Fig. 3).

### 3.5 Correlation analysis of different variables in the two patient groups

In the RLIC group, the MoCA score was negatively correlated with P300 latency and positively correlated with amplitude, with Pearson correlation R of -0.603 ( $P < 0.05$ ) and 0.476 ( $P < 0.05$ ), respectively. Specifically, the latency of P300 was negatively cor-



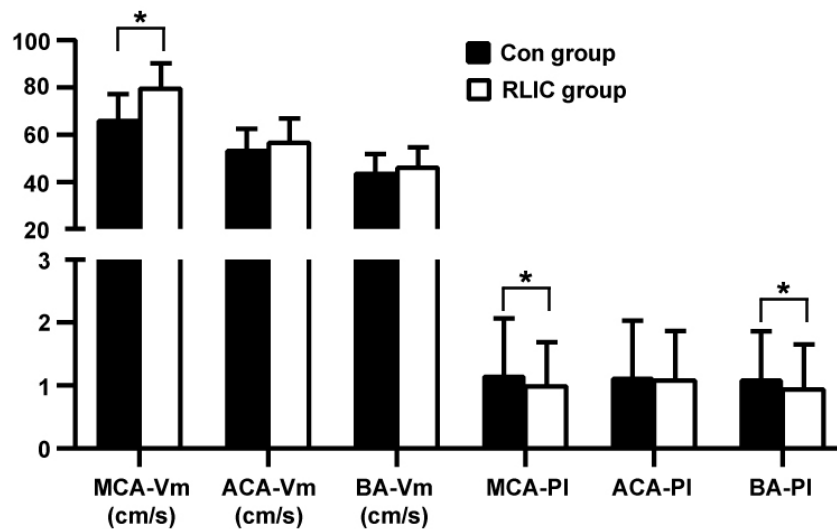


Figure 3. Blood flow indices at 6 months poststroke (\* indicate  $P < 0.05$ ): MCA-Vm was significantly higher in the RLIC group than in the Con group ( $P = 0.035$ ), while both MCA-PI ( $P = 0.019$ ) and BA-PI ( $P = 0.021$ ) were significantly lower in the RLIC group than in the Con group.

related with MoCA domains of visuospatial and executive functioning and attention scores ( $R = -0.442$ ,  $P < 0.05$ ;  $R = -0.515$ ,  $P < 0.05$ , respectively). P300 amplitude was positively correlated with MoCA domains of visuospatial and executive functioning and attention score ( $R = 0.495$ ,  $P < 0.05$ , and  $R = 0.576$ ,  $P < 0.05$ , respectively).

MCA-PI measurement was positively correlated with ICAM-1 and ET-1 ( $R = 0.390$ ,  $P < 0.05$ , and  $R = 0.542$ ,  $P < 0.05$ , respectively). BA-PI was also positively correlated with ICAM-1 and ET-1 levels ( $R = 0.309$ ,  $P < 0.05$  and  $R = 0.451$ ,  $P < 0.05$  respectively). ICAM-1 and ET-1 were, in turn, positively correlated with baPWV ( $R = 0.407$ ,  $P < 0.05$  and  $R = 0.530$ ,  $P < 0.05$ , respectively).

In the control group, the MoCA score was also negatively correlated with P300 latency and positively correlated amplitude, with Pearson Correlation ( $R$ ), of  $-0.506$  ( $P < 0.05$ ), and  $0.537$  ( $P < 0.05$ ), respectively. The latency of P300 was negatively correlated with MoCA domains of visuospatial and executive functioning and attention scores ( $R = -0.397$ ,  $P < 0.05$ , and  $R = -0.452$ ,  $P < 0.05$ , respectively). P300 amplitude was positively correlated with MoCA domains of visuospatial and executive functioning and attention scores ( $R = 0.470$ ,  $P < 0.05$ , and  $R = 0.525$ ,  $P < 0.05$ , respectively). MCA-PI was positively correlated with ICAM-1 and ET-1 levels ( $R = 0.410$ ,  $P < 0.05$  and  $R = 0.490$ ,  $P < 0.05$ , respectively). MCA-Vm was negatively correlated with ICAM-1 and ET-1 levels ( $R = -0.353$ ,  $P < 0.05$  and  $R = -0.397$ ,  $P < 0.05$ , respectively). Finally, ET-1 was positively correlated with baPWV ( $R = 0.435$ ,  $P < 0.05$ ).

#### 4. Discussion

Early recognition and intervention against PSCI in stroke patients are crucial. It is known that 17-92% of stroke patients experience mild cognitive impairment within 3 months, while 6-32% of patients with PSCI progress to dementia (Pasi et al., 2012). While the specific rate of incidence of PSCI may vary in different countries, regions, ethnicities, and diagnostic systems, there is scien-

tific consensus that the risk of PSCI remains very high in stroke patients and is being observed to rise (Pendlebury and Rothwell, 2009; Qu et al., 2015; Sun et al., 2014; Yu et al., 2013). PSCI detrimentally impacts patient compliance in physical rehabilitation training, severely hindering limb function restoration and reducing the quality of life and survival of the patient.

Remote limb ischemic conditioning is a nonpharmacological, noninvasive treatment that promises clinical transformation (Meng et al., 2015). Its biggest advantages are the absence of definite adverse effects and a greater chance of patient acceptance. In a recent clinical trial, Hougard et al. (2013) investigated the feasibility of RLIC in prehospital rescue for patients with acute ischemic stroke and found that RLIC remarkably reduced the risk of cerebral infarct. Further, the Remote Ischemic Conditioning After Stroke Trial study assessed patients within 24 hours of an ischemic stroke who received four cycles of RLIC. Their findings revealed that RLIC improved patients' National Institute of Health stroke scale (NIHSS) scores in 90 days (England et al., 2017). It has also been demonstrated that brief, repetitive, bilateral arm ischemic preconditioning combined with current standard medical management can reduce stroke recurrence in patients ( $< 80$  years) with symptomatic atherosclerotic intracranial arterial stenosis (SIAS) (Meng et al., 2012).

Moreover, it significantly improved cerebral perfusion and metabolism and reduced focal vascular resistance in the stenotic area. In a later study, the team found that RLIC was also safe and well-tolerated in elderly patients with SIAS ( $> 80$  years) who were not candidates for endovascular interventions, while RLIC could lower the recurrence rate of ischemic events (stroke and transient ischemic attack) (Meng et al., 2012). Together, these studies proved that RLIC is a safe, feasible, and remarkably efficacious intervention for cerebral infarct prevention.

Currently, most studies on RLIC in vascular dementia have been limited to animal experiments. The bilateral common carotid artery occlusion model in rodents is considered the most valid animal model for simulating vascular cognitive impairment (VCI). In

a study by [Xu et al. \(2011\)](#), 36 rats randomized into control group, bilateral carotid arteries occlusion group, and RLIC group, determined that neurocognitive function was significantly improved in RLIC group rats as compared to the occlusion model rats in 7-8 days ([Xu et al., 2011](#)). Interestingly, they found that RLIC could increase the expression of Bcl-2, reduce apoptosis of nerve cells, and improve nerve function. Interestingly, RLIC has also been suggested to improve cognitive function in cerebrovascular impairment via the induction of autophagy and increased expression of autophagy-related proteins ([Wang et al., 2017b](#)).

On the other hand, [Khan et al. \(2015\)](#) found that RLIC could reduce cognitive impairment via improved cerebral blood flow and reduced tissue injury in mice with vascular cognitive impairment (VCI). Additional evidence also points to the role of RLIC in improving cognitive impairment in patients following colon surgery and heart surgery ([He et al., 2017](#); [Hudetz et al., 2015](#)). [Wang et al. \(2017b\)](#) applied a five-cycle RLIC to patients with cerebral small-vessel disease (cSVD) twice daily for 1 year and found that the treatment could decelerate cognitive function decline in patients with cSVD. Most importantly, no severe adverse events were reported following RLIC in any of these studies.

Under current standards, neuropsychiatric evaluation remains an important method for the recognition and diagnosis of cognitive impairment and a crucial tool for monitoring treatment efficacy and disease outcome. In PSCI screening, the MoCA scale is more sensitive than the Mini-Mental State Examination (MMSE) and is considered to have higher reliability and validity ([Blackburn et al., 2013](#); [Cumming et al., 2013](#)). Our study has found that the MoCA score was higher, and the ADL score lower in the RLIC group than control group patients at 6 months poststroke. Specifically, MoCA scores were significantly higher in the domains of visuospatial and executive functioning, and attention, in RLIC group patients. Additionally, the incidence of PSCI was significantly lower in the RLIC group than in the control group. Overall, these results strongly indicate that RLIC improved cognitive function in patients following stroke and suggest its role as a key prevention strategy against PSCI.

P300 event-related potential is a sensitive test for specific cognitive activities, and its major monitoring indices are latency and amplitude. P300 latency is negatively correlated with the MMSE score, with shorter latencies associated with superior cognitive performance. Therefore, P300 latency can aid in the determination of a decline in cognitive function and therapeutic efficacy, serving as an effective clinical tool. Correlation analyses performed in our study consistently indicated that patients' MoCA scores were negatively correlated with P300 latency and positively correlated with amplitude, respectively. These findings suggest a relationship between severe cognitive impairment, longer P300 latency, and lower P300 amplitude.

Further, P300 latency was negatively correlated with MoCA scores in the domains of visuospatial and executive functioning and attention, while P300 amplitude was positively correlated with these scores. These results thus suggest a link between severe damage in the stated domains with longer latency times and lower amplitudes. Together, our observation of significantly shorter P300 latency and significantly higher P300 amplitude in RLIC group patients as compared to control group patients, further proves that

RLIC improves cognitive function following stroke.

The neuroprotective mechanisms of RLIC are complex, involving multiple factors and signaling pathways, the precise details of which are still poorly understood. RLIC treatment alters the expression of multiple genes and proteins, including hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), HIF-1 $\beta$ , the anti-apoptotic gene Bcl-2, and superoxide dismutase (SOD), all of which are observed to be elevated. RLIC has also been observed to inhibit the expression of the apoptotic protein, p53 ([Jin et al., 2016](#); [Salido et al., 2013](#); [Tidball et al., 2016](#)). These changes can affect a range of processes, such as improving the metabolism of nerve cells, inhibiting oxidative damage, and reducing neuronal apoptosis. The use of single-photon emission CT to evaluate the impact of RLIC on metabolism and blood flow restoration showed significantly increased blood flow at affected brain sites ([Meng et al., 2012](#)). This radiographically verified the correlation between the cerebral protective effects of RLIC and increased cerebral perfusion in the ischemic region. Our study similarly used TCD detection technique to assess cerebral blood flow to find that RLIC indeed increases mean blood flow in the middle cerebral artery and reduces cerebral artery and basilar artery PI, thereby improving the blood supply to the brain.

ICAM-1 is a key adhesion molecule for leukocytes crossing the blood-brain barrier (BBB). ICAM-1 expression is observed to significantly increase following cerebral ischemia. Leukocytes adhere to endothelial cells, transmigrate, and infiltrate into the extravascular brain parenchyma, becoming concentrated in regions of injury due to the binding of ICAM-1 proteins with corresponding receptors on leukocytes. This results in microvascular occlusion and production of oxygen-free radicals. In turn, cytotoxic enzymes are released, increasing BBB permeability, altering the vasoconstriction response, stimulating the release of inflammatory cytokines, and causing brain tissue damage. Meanwhile, microvascular occlusion aggravates cerebral ischemia, setting up a vicious cycle ([Cheng et al., 2008](#); [Shen et al., 2008](#)).

As the most potent vasoconstrictor so far ([Kwan et al., 2002](#)), ET-1 is synthesized in vascular endothelial cells, smooth muscle cells, neurons, and glial cells, while its receptors are readily available in the cerebrovascular system. Cerebral ischemia and hypoxic conditions greatly increase the production and release of ET-1. This results in atherosclerosis due to the promotion of vascular smooth muscle proliferation and aggravated inflammation from enhanced neutrophil adhesion. These changes cause considerable damages to nerve cells and vascular endothelial cells, directly impacting synaptic plasticity and injuring the cholinergic system, and therefore affecting cognitive function ([Masaki et al., 1991](#); [Selnes and Vinters, 2006](#)). Serum ET-1 levels are strongly negatively correlated with cognitive impairments ([Briyal et al., 2011](#)).

Serum ICAM-1 and ET-1 are also important markers of endothelial function ([Armentero et al., 2011](#); [Fang et al., 2011](#)). Vascular endothelial cells regulate blood flow, control the permeability of plasma components, and influence the adhesion and aggregation of platelets and cytokines. Therefore, a healthy endothelium plays an important role in the regulation of cerebral vascular homeostasis ([Michiels, 2003](#)). The pathophysiological effects of vascular endothelial dysfunction on atherosclerosis have been confirmed by numerous studies ([Yoshida et al., 2010](#)). In the present study, we have also found that RLIC can reduce serum levels of

ICAM-1 and ET-1 and improve endothelial function. We speculate that this favorable effect may be associated with the fact that RLIC improves blood supply to the brain and alleviates cerebral ischemia and hypoxia, which, in turn, reduces serum levels of ICAM-1 and ET-1. Pearson correlation analysis also suggested that the improvement of cerebral hemodynamics may also improve vascular endothelial function.

Finally, baPWV is also a sensitive index for the assessment of atherosclerosis (Sabayan et al., 2013). Increased arterial stiffness can lead to a decrease in arterial buffering function, while increased severity of cerebrovascular injury, cerebrovascular damage, and cerebral hypoperfusion may result in damage to neurons in the vulnerable areas of the brain (such as hippocampal gyrus neurons), leading to cognitive decline (Cavalcante et al., 2011; Najjar et al., 2008; O'Sullivan et al., 2003; Rothwell et al., 2010). In our study, RLIC was able to decelerate baPWV and reduce the degree of arteriosclerosis. This effect might also be associated with a reduction in ICAM-1 and ET-1 levels and improvement in vascular endothelial cell function following RLIC. Pearson correlation analyses on our data consistently suggested that vascular endothelial function can slow baPWV and reduce the degree of arteriosclerosis.

In conclusion, RLIC improved blood supply to the brain and endothelial function, alleviated degrees of arteriosclerosis, improved cognitive function following stroke, and reduced the incidence of PSCI. It especially improved cognitive domains linked to visuospatial, executive functioning, and attention. As such, RLIC can serve as an effective clinical strategy for PSCI improvement.

## Ethics approval and consent to participants

This research was approved by the ethical review board of Zhabei Central Hospital, Jing'an District, Shanghai, People's Republic of China, code ZBL2018062605. All participants gave written consent after being informed about the experimental procedures.

## Author contributions

S.X. conceived this project and supervised the experiments. X.F. and S.X. wrote the paper. X.F., L.H., Z.W., L.W., X.D., Q.W., and S.X. performed the experiments and analyzed the data.

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## Conflict of interest

The authors declare no conflict of interest.

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