Potential Anti-Inflammatory and Anti-Coagulation Effects of One-Time Application of Remote Ischemic Conditioning in Patients With Subacute/Chronic Cerebral Arteriostenosis and Venostenosis

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Background: Remote ischemic conditioning (RIC) is an extremely simple, non-invasive, and cost-effective method with a neuroprotective effect. This study aimed to evaluate the immediate effects of one-time application of RIC on inflammation and coagulation in patients with chronic cerebral vascular stenosis, and compare the different effects of RIC on cerebral arteriostenosis and cerebral venostenosis.

Method: A total of 47 patients with defined cerebral arteriostenosis (n = 21) or venostenosis (n = 26) were prospectively enrolled. RIC intervention was given once with 5 cycles of inflating and deflating for 5 minutes alternately. Blood was sampled 5 minutes before and after RIC for inflammatory and thrombophilia biomarkers. Differences in inflammatory and thrombotic variables at differing time points in the group were assessed using paired t tests or Wilcoxon matched-pairs signed-rank test.

Results: Patients with cerebral arteriostenosis had a higher level of pre-RIC neutrophil-to-lymphocyte ratio (P = 0.034), high-sensitivity C-reactive protein (P = 0.037), and fibrinogen (P = 0.002) than that with cerebral venostenosis. In the arterial group, levels of fibrinogen (P = 0.023) decreased, and interleukin-6 levels were elevated (P = 0.019) after a single RIC. Age was negatively related to interleukin-6, C-reactive protein, and fibrinogen.

Conclusion: One-time RIC interventions may show seemingly coexisted proinflammatory and anti-coagulation effects of a single bout on patients with cerebral arteriostenosis. Older age was a negative predictor for multiple biomarkers in the cerebral arteriostenosis group. The protective effect of RIC on cerebral venostenosis patients needs to be further studied in a larger sample size.

Key Words: remote ischemic conditioning, cerebral arteriostenosis, cerebral venostenosis, anti-inflammation, anti-coagulation

Remote ischemic conditioning (RIC), as an extremely simple, noninvasive, and cost-effective method, has been proven to have multiorgan protective effects, especially in renal,1 cardiological,3 and neurological system,4,5 based on solid evidence from both experimental and clinical studies. RIC entails induction of brief cycles of relative ischemia and reperfusion in a targeted area to prevent end organ injury due to ischemia in distant tissues. Human responses to RIC-stimuli are generally thought to occur in 2 separate timeframes.6,7 The first (acute) response lasts between 2 and 4 hour, and mainly presents with acute gains in cutaneous microcirculation through NO-synthesis enhancements.8,9 A second window of protection appears to open 12 and 24 hours after the RIC-stimulus. Protective measures within this phase can be attributed to 3 major signal transmission pathways,4 including the humoral pathway (which is mediated by nitric oxides, nitrite, and SDF-1α), the immunological pathway [which is mediated by downregulating proinflammatory markers, including interleukin (IL)-6 and IL-1] and the neurological pathway (which is mediated by direct stimulation of peripheral sensory nerves).

Our team firstly demonstrated the positive prognostic effect of long-term RIC in the rhesus monkey model of ischemic stroke and patients with acute ischemic stroke after thrombectomy,10 intracranial atherosclerotic stenosis,11,12 and cerebral small vessel disease.13 Besides, we further illustrated the underlying mechanisms that RIC could decrease stroke recurrence and white matter lesions by improving cerebral perfusion, reducing inflammation via decreasing C-reactive protein (CRP), interleukin-6 (IL-6), leukocyte count, and inhibiting the coagulation process through decreasing
arteriostenosis and cerebral venostenosis. Moreover, although the efficacy and safety of RIC in cerebral vascular disease have been reported, personalized RIC intervention regimes need to be considered because patients tended to question whether this new adjunctive strategy would benefit their health situation when they made the decision to use it in the long term. Several recent proteomic analyses in healthy rat models and humans have shown that RIC stimulus can cause early global proteomic responses at 5, 10, and 15 minutes post singular-time RIC. Proinflammatory peptides are predominantly down-regulated—and anti-inflammatory peptides predominantly up-regulated—in these early responses. Based on these previous findings, we speculated that, following one-time RIC applications, if patients showed significant regulation of inflammatory or coagulation markers, they may benefit from long-term RIC interventions. Further examination of this speculation may shed light on personalized RIC intervention regimes and help physicians and patients to make better decisions. Thus, in this study, we aimed to evaluate the immediate effects of one-time RIC application on inflammation and coagulation in patients with subacute/chronic cerebral vascular stenosis, and, for the first time, to compare the differential effects of RIC on cerebral arteriostenosis and cerebral venostenosis.

METHODS

Population
A total of 59 patients with suspected subacute/chronic cerebral arteriostenosis or venostenosis were prospectively enrolled in the Department of Neurology, Xuanwu Hospital, Capital Medical University, between 2018 and 2020. This study was approved by the Ethics Committee of the Xuanwu Hospital at Capital Medical University. All participants signed the consent form before entering this study.

We included certain patients who were symptomatic even after long-term lifestyle modifications, standardized antiplatelet/anticoagulant therapies, and/or had well-controlled hypertension/diabetes/hyperlipidemia. The common symptoms of patients with symptomatic cerebral arteriostenosis usually presented with chronic headache or dizziness, and the common symptoms of patients with symptomatic cerebral venostenosis were chronic headache and tinnitus. The symptomatic course at administration was defined as subacute phase (2 wk to 6 mo) and chronic phase (more than 6 mo).

The included patients were originally enrolled in the outpatient setting, and then, after serious radiologic and laboratory workup, only those with defined cerebral arteriostenosis or venostenosis [either cerebral venous sinus stenosis (CVSS) or internal jugular venous stenosis (IJVS)] and without new-onset ischemic or hemorrhagic lesion in the brain were eventually included. Cerebral stenosis was examined by ultrasound, contrast-enhanced magnetic resonance arteriography/venography, computed tomography arteriography/venography, or digital subtraction angiography.

For evaluation of the degree of cerebral arteriostenosis by Doppler studies, low-grade stenosis was defined as a slight to moderate increase in local flow and a 50% to 70% diameter reduction in duplex imaging; high-grade cases were classified as >70% diameter reduction; subtotal stenosis was with very narrow stenosis and low flow velocities at the maximum narrowing; occlusion described the status of no detectable cerebral vessel flow. We evaluated major vessels in both the anterior and posterior cerebral circulation, including internal carotid artery, anterior cerebral artery, middle cerebral artery, posterior cerebral artery, vertebral artery, basilar artery, and superior cerebellar artery. The severity grade of stenosis was defined by the degree of the most seriously affected vessel. The location of predominant cerebral stenosis was defined after clinical and radiologic evaluation, based on clinical manifestation and neuroimaging of which vessel was most seriously affected and how many stenotic vessels were, respectively, involved in anterior or posterior circulation.

For the evaluation of cerebral venostenosis (CVSS and IJVS), first, patients were noninvasively screened through transcranial and extracranial echo-color Doppler ultrasound based on venous hemodynamic criteria. A subject is considered cerebral venostenosis positive if more than 2 of 5 characteristics are fulfilled. Then, the confirmed diagnosis of cerebral venostenosis was made by contrast-enhanced magnetic resonance venography or digital subtraction angiography. The major evaluated veins included superior sagittal sinus, transverse sinus, straight sinus, sigmoid sinus, and internal jugular vein.

Exclusion criteria were as following: (1) definite acute or chronic infection; (2) definite hematological disease (eg, thrombophilia, anemia, leukopenia, and leukemia/lymphoma); (3) use of anti-inflammatory medication within 4 weeks before blood collection; (4) ongoing menstrual period, oral contraceptive use or pregnancy (for female patients); (5) definite subclavian steal syndrome; (6) definite new-onset ischemic/hemorrhagic lesion.

All study participants served as their own controls and were studied twice: once at study enrollment and once right after completing their RIC treatment.

Clinical and Demographic Data

Age, sex, symptomatic course at admission (spanning from symptom onset to admission), treatments, and presumably risk factors known before hospitalization or discovered during hospitalization were recorded (Table 1). The common risk factors included hypertension (use of antihypertensive medications or systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg before hospitalization diastolic blood pressure >90 mm Hg before hospitalization), diabetes mellitus (use of antidiabetic therapies or fasting blood glucose >7 mmol/L), a history of myocardial infarction or angina, overweight (body mass index >25 kg/m²), previous anemia with hemoglobin <12.5 g/dL, HBV infection (use of anti HBV therapies or positive HBeAg/Ag or HBsAg), hyperhomocysteinemia (>15 mmol/L), hyperuricemia (>416 mmol/L), chronic rhinosinusitis, history of otitis media/mastoiditis, suspected thyroid disorders (including either abnormal thyroid ultrasound results or abnormal thyroid function results), autoimmune disease, thrombophilia (including protein S deficiency, protein C deficiency, antithrombin III deficiency, hyperfibrinogenemia, primary thrombocytopenia or increased D-dimer level), and history of ischemic or hemorrhagic stroke.

Subgroup analysis was conducted based on disease entity, including A group (defined as a group of patients with cerebral arteriostenosis) and V group (defined as a group of patients with cerebral venostenosis).
RIC intervention was performed by an auto-control blood pressure cuff on bilateral upper limbs (Fig. 1A, patent number ZL200820123637.X, China). This device could automatically inflate or deflate the bilateral cuffs, to mimic brief cycles of relative ischemia and reperfusion in upper limbs. The default max compression pressure was 180 mm Hg. A whole RIC intervention session consisted of 5 cycles of inflating and deflating for 5 minutes alternately (in total 50 min per treatment). The enrolled patients underwent a single time of RIC intervention in this study. This intervention process and devices of RIC intervention were visualized in Figures 1A and B.

Routine monitoring during RIC included blood pressure, heart rate, respiration, as well as the visual analog scale of pain. The RIC intervention would be terminated if a patient could not tolerate and withdrawn from the study. Meanwhile, routine treatment, such as antiplatelet or anticoagulation therapy, was as same as the traditional medical treatment prior to RIC.

**Blood Sampling**

The schedule for blood sampling was demonstrated in Figure 1A. All participants were required for no alcohol/caffeine intake for 24 hours and no consumption of food or liquids within the last hour before blood sampling. First, participants rested for 30 minutes before any procedures. Blood was withdrawn before RIC intervention (baseline, pre-RIC) and 5 minutes after the intervention.

**Inflammatory and Thrombophilia Biomarkers Assay in the Blood Sample**

Inflammatory biomarkers assay included neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) in plasma EDTA samples, as well as IL-6, CRP, hypersensitive CRP (hs-CRP), and procalcitonin (PCT) in serum samples. Baseline levels were measured on admission. NLR was computed using the absolute neutrophil count divided by the absolute lymphocyte count. PLR was calculated using the absolute platelet count divided by the absolute lymphocyte count. Baseline inflammatory markers were considered as continuous variables.

Thrombophilia biomarkers assay evaluated both antigens, including platelet (PLT), fibrinogen, D-dimer (DD), antithrombin-III (AT-III), protein C (PC), and protein S (PS) in plasma sodium-citrate sample without platelet depleted.

All blood samples were collected in VACUETTE Blood Collection Tubes (Greiner Bio-One, Kremsmünster, Austria).

**TABLE 1. Demographic and Basic Clinical Features**

| Variables                              | All (n = 47) | A Group (n = 21) | V Group (n = 26) | P      |
|----------------------------------------|-------------|-----------------|-----------------|--------|
| **Personal data**                      |             |                 |                 |        |
| Age*                                   | 49.49 ± 15.64 | 56.76 ± 11.70 | 43.62 ± 16.13   | 0.006  |
| Sex (M:F)                              | 25:22       | 15:6            | 10:16           | 0.039  |
| BMI*                                   | 26.14 ± 4.27 | 26.07 ± 3.46   | 26.20 ± 4.89    | 0.724  |
| **Symptomatic course at admission, n (%)** |             |                 |                 |        |
| Subacute (2 wk to 6 mo)                | 14 (29.8)   | 9 (42.9)        | 5 (19.2)        | 0.112  |
| Chronic (more than 6 mo)               | 33 (70.2)   | 12 (25.5)       | 21 (80.8)       |        |
| **Presumable risk factors, n (%)**     |             |                 |                 |        |
| Obesity (BMI > 25)                     | 24 (51.1)   | 12 (25.5)       | 12 (46.2)       | 0.561  |
| Type 2 DM                              | 8 (17.0)    | 5 (23.8)        | 3 (11.5)        | 0.437  |
| HBP                                    | 28 (59.6)   | 18 (85.7)       | 10 (38.5)       | 0.001  |
| Hyperlipidemia                         | 30 (63.8)   | 15 (71.4)       | 15 (57.7)       | 0.375  |
| Anemia                                 | 5 (10.6)    | 1 (4.8)         | 4 (15.4)        | 0.362  |
| IS/TIA                                 | 24 (51.1)   | 17 (81.0)       | 7 (26.9)        | <0.001 |
| ICH                                     | 4 (8.5)     | 1 (4.8)         | 3 (11.5)        | 0.617  |
| Hyperuricemia                          | 5 (10.6)    | 1 (4.8)         | 4 (15.4)        | 0.362  |
| Hyperhomocysteinemia                   | 5 (10.6)    | 1 (4.8)         | 4 (15.4)        | 0.369  |
| CAD                                    | 5 (10.6)    | 4 (19.0)        | 1 (3.8)         | 0.158  |
| Previous otitis media/mastoiditis      | 3           | NA              | 3 (11.5)        | NA     |
| Chronic rhinosinusitis                 | 4 (8.5)     | NA              | 4 (15.4)        | NA     |
| HBV infection                         | 1 (2.1)     | NA              | 1 (3.8)         | NA     |
| Suspected thyroid disorders, n (%)     |             |                 |                 |        |
| Abnormal thyroid ultrasound            | 4 (8.5)     | 2 (9.5)         | 2 (7.7)         | 1.000  |
| Abnormal thyroid function test         | 11 (23.4)   | 3 (15.3)        | 8 (30.8)        | 0.300  |
| **Treatment, n (%)**                   |             |                 |                 |        |
| Antiplatelet drugs                     | 29 (61.7)   | 18 (85.7)       | 11 (42.3)       | 0.003  |
| Aspirin                                | 22 (46.8)   | 12 (52.5)       | 6 (23.1)        | 0.020  |
| Clopidogrel                            | 18 (38.3)   | 12 (52.5)       | 6 (23.1)        | 0.033  |
| Anticoagulants                         | 24 (51.1)   | 3 (15.3)        | 21 (80.8)       | 0.000  |
| LMWH                                   | 8 (17.0)    | NA              | 8 (30.8)        | NA     |
| Dabigratran                           | 3 (6.4)     | NA              | 3 (11.5)        | NA     |
| Rivaroxaban                            | 14 (29.8)   | 1 (4.8)         | 13 (50.0)       | 0.001  |
| Endovascular therapies                 | 5 (10.6)    | NA              | 5 (19.2)        | NA     |
| Stenting                               | 4 (8.5)     | NA              | 4 (15.4)        | NA     |
| Balloon dilation                       | 1 (2.1)     | NA              | 1 (3.8)         | NA     |
| ONSD, n (%)                            | 2 (4.3)     | NA              | 2 (7.7)         |        |

*Mean ± SD.

Bold values indicate statistically significant.

*CAD indicates coronary artery disease; DM, diabetes mellitus; F, female; HBP, high blood pressure; HBV, hepatic type B virus; ICH, intracranial hemorrhage; IS, ischemic stroke; LMWH, low-molecular-weight heparin; M, male; NA, not applicable; ONSD, optic nerve sheath decompression; TIA, transient ischemic attack.
Statistical Analysis

Continuous data following a Gaussian distribution is presented as mean ± SD and categorical data is expressed as n (%). T test or Fisher exact test to compare continuous variables or categorical variables between patients with cerebral arteriostenosis and cerebral venostenosis. Wilcoxon Signed Rank can be used as an alternative to the t test when the population data does not follow a normal distribution. Differences in inflammatory and thrombotic variables at differing time points in the group were assessed using paired t tests or Wilcoxon matched-pairs signed-rank test. Multilinear analysis was conducted to analyze predictors of pre-RIC inflammatory markers. Stata software (version 15.0 SE) and R software [version 3.6.2 (2019-12-12)] was used in this study for all analyses. Two-sided P-values < 0.05 were considered significant.

Data Availability

Anonymized data used here can be shared by request to any qualified investigators.

RESULTS

Baseline Clinical Features

Figure 2 displayed the enrollment process, eventually with a total of 47 patients included in this prospective study. Among them, 21 patients were with isolated cerebral arteriostenosis and 26 patients were with isolated cerebral venostenosis. More than 70% had a disease course over 1 month (chronic stage) (n = 33). The average body mass index was over 26, which indicated a high prevalence of obesity in this cohort. The most common presumable risk factors were hyperlipidemia (63.8%), hypertension (59.6%), and previous ischemic stroke/transient ischemic attack history (51.1%) (Table 1).

Clinical Difference Between Cerebral Arteriostenosis and Cerebral Venostenosis

Cerebral arteriostenosis were more prone to present in male and older population than cerebral venostenosis. The traditional vascular risk factors, such as hypertension (87.5%, P = 0.001) and previous ischemic stroke/transient ischemic attack history (81.0%, P < 0.001), were more likely to be associated with cerebral arteriostenosis. Moreover, over 80% of patients with arteriostenosis underwent antiplatelet therapy, while patients with venostenosis were treated with anticoagulants, especially novel oral anticoagulants, such as Rivaroxaban and Dabigatran (Table 1).

Fifteen patients had predominant stenosis involved in cerebral anterior circulation, while 6 patients were mostly affected in posterior circulation. More than 80% of patients were suffered from severe stenosis, including high grade (n = 5), subtotal stenosis (n = 5), and occlusions (n = 3). Anterior cerebral artery, middle cerebral artery, and VA were the top 3 most likely involved cerebral arterial vessels (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/NRL/A78). In group of cerebral venostenosis, 12 patients presented with CVSS combined with IJVS, 10 patients with CVSS, and 4 patients with IJVS. CVSS was most likely involved in multiple sites at the same time, most commonly caused by thrombus in situ. However, IJVS was mostly due to bone compression at the J3 segment (Supplementary Table 2, Supplemental Digital Content 2, http://links.lww.com/NRL/A79).

Inflammatory and Thrombophilia Biomarkers Change After RIC Intervention

Before RIC treatment, patients with cerebral arteriostenosis had elevated CRP, hs-CRP, and DD levels compared with normal range, which may indicate hyperinflammatory and hypercoagulable state. However, all inflammatory and thrombophilia biomarkers were within normal ranges in patients with cerebral venostenosis. Besides, A group had a higher level of pre-NLR (P = 0.034), hs-CRP (P = 0.037), and fibrinogen (P = 0.002) than V group (Table 2, Fig. 3).
Then, we also evaluated the change of inflammatory and thrombophilia biomarkers after RIC treatment. Intriguingly, no significant difference between pre-RIC values and post-RIC values was found in V group. In A group, the post-RIC fibrinogen level was slightly lower than the pre-RIC fibrinogen level ($P = 0.023$) (Table 2, Fig. 3). Moreover, post-RIC NLR and PLR levels were decreased compared with the baseline levels despite no statistical significance, which might be due to the small sample size. However, these results at least gave us some hint that even short-term RIC may potentially have an immediate effect of anti-inflammation and anti-coagulation, which may be helpful in emergency settings (Table 2).

**Probable Predictors of Pre-RIC Inflammatory Biomarkers in Patients With Cerebral Arteriostenosis**

As we found in Table 2 that patients with cerebral arteriostenosis are more likely to have higher inflammatory and thrombophilia biomarkers, and benefit from RIC treatment, we further conducted multilinear regress analysis to evaluate the probable predictors of inflammatory and coagulation state. Before RIC treatment, older age was correlated with higher red blood cell distribution width (RDW) ($P = 0.003$), IL-6 ($P = 0.024$), CRP ($P = 0.038$), and fibrinogen ($P = 0.018$) level. Occluded vessel may attribute to elevated NLR level ($P = 0.018$). Location of predominant cerebral arteriostenosis (posterior circulation or anterior circulation) and sex were not indicative of inflammatory (Table 3) and thrombophilia markers (Table 4). After RIC treatment, age was the positive predictor of RDW, IL-6, and CRP (Supplementary Table 3A, Supplemental Digital Content 3, http://links.lww.com/NRL/A80). Subtotal stenosis of vessels was related to decreased AT-III (Supplementary Table 3B, Supplemental Digital Content 3, http://links.lww.com/NRL/A80).

**DISCUSSION**

In this study, we found that patients with cerebral arteriostenosis had a higher level of pre-RIC CRP, hs-CRP, and DD than normal upper limits and the group of cerebral venostenosis, while patients with cerebral venostenosis had all
inflammatory and thrombophilia biomarkers within the normal range. After a singular RIC intervention, fibrinogen and AT-III levels decreased in the arterial group. Post-RIC NLR and PLR levels also showed decreases, but the changes did not reach statistical significance. However, interestingly, post-RIC IL-6 was significantly elevated compared to pre-RIC levels. This may indicate that immediate RIC-related effects could be involved in inflammatory and coagulation processes. Moreover, older age was found as a negative predictor for multiple biomarkers, such as RDW, IL-6, CRP, and fibrinogen.

In our knowledge, this is the first study to evaluate patients with cerebral venostenosis after a single bout of RIC intervention, as well as compare the difference of pre/post-RIC inflammatory and thrombophilia biomarkers levels between groups of cerebral arteriostenosis and venostenosis. Although no significant effect of short-term RIC was revealed in a group of venostenosis in terms of inflammation and coagulation, further study was suggested to apply long-term RIC to this population because we recently found that cerebral venostenosis decreased cerebral perfusion and then potentially co-presented with cerebral arteriostenosis.

Two disease entities may share common pathologic mechanisms.

Intriguingly, another novel finding in this study was the seemingly coexisted proinflammatory (increased IL-6 level) and anti-coagulation (decreased fibrinogen level) effect of singular time RIC on patients with cerebral arteriostenosis. This is consistent with the results of several former animal studies. Yang and his colleagues indicated that application of singular time RIC increased the shift of circulating monocytes to CCR2+ proinflammatory subset and eventually benefited stroke recovery in mice model of acute ischemic stroke. They found that once-daily RIC for 1 week promoted delayed hematoma resolution via macrophage activation and improved outcome after spontaneous intracerebral hemorrhage in mice. Moreover, as reviewed by Landman et al, both a single bout of RIC and repeated RIC was proved to have a protective effect on distant organs in preclinical and clinical studies. Our finding may bring practical biomarkers for physicians to assess the probable benefit of long-term RIC on every single patient after only one RIC intervention.

Application of singular time RIC consistently increased the shifts of circulating monocytes to CCR2+ proinflammatory subset and eventually benefited stroke recovery in mice model of acute ischemic stroke. Vaibhav et al found that once-daily RIC for 1 week promoted delayed hematoma resolution via macrophage activation and improved outcome after spontaneous intracerebral hemorrhage in mice. Moreover, as reviewed by Landman et al, both a single bout of RIC and repeated RIC was proved to have a protective effect on distant organs in preclinical and clinical studies. Our finding may bring practical biomarkers for physicians to assess the probable benefit of long-term RIC on every single patient after only one RIC intervention.

There are well-known age-related effects (ie, effects among patients older than 65) on inflammation (eg, elevated CRP and IL-6), fibrinogen level) effect of singular time RIC on patients with cerebral arteriostenosis. This is consistent with the results of several former animal studies. Yang and his colleagues indicated that application of singular time RIC increased the shift of circulating monocytes to CCR2+ proinflammatory subset and eventually benefited stroke recovery in mice model of acute ischemic stroke. Vaibhav et al found that once-daily RIC for 1 week promoted delayed hematoma resolution via macrophage activation and improved outcome after spontaneous intracerebral hemorrhage in mice. Moreover, as reviewed by Landman et al, both a single bout of RIC and repeated RIC was proved to have a protective effect on distant organs in preclinical and clinical studies. Our finding may bring practical biomarkers for physicians to assess the probable benefit of long-term RIC on every single patient after only one RIC intervention.

There are well-known age-related effects (ie, effects among patients older than 65) on inflammation (eg, elevated CRP and IL-6). In this study, mean ages of both groups (arterial group: 56.76 ± 11.70; venous group: 43.62 ± 16.13) were lower than 65. In addition, both groups were at subacute/chronic disease stages. Thus, we expected them to have normal levels of inflammatory markers. However, we found that older age was a negative predictor for multiple biomarkers, including RDW, IL-6, CRP, and fibrinogen, at least in patients in the arterial group who had short-term RIC. Our former study demonstrated that octogenarians and nonagenarians with symptomatic intracranial arterial stenosis had a
TABLE 3. Multivariate Linear Regression Analysis of Pre-RIC Inflammatory Biomarkers in Patients With Cerebral Arteriostenosis (n = 21)

| Predictors                                         | Pre-RIC | NLR   | PLR   | RDW   | IL-6   | CRP   | hs-CRP | PCT   |
|----------------------------------------------------|---------|-------|-------|-------|--------|-------|--------|-------|
| Age (y)                                            | 0.01 (0.02) | 0.90 (1.22) | 0.03 (0.01)** | 1.05 (0.42)* | 2.11 (0.92)* | 0.54 (0.28) | 0.0 (0.00) |
| Sex (male vs. female)                              | 0.44 (0.62) | 3.85 (35.41) | 0.47 (0.28) | -0.01 (12.06) | 1.15 (26.86) | 1.02 (8.24) | -0.04 (0.06) |
| Location of predominant cerebral arteriostenosis (posterior vs. anterior) | 0.71 (0.62) | 36.29 (35.63) | 0.36 (0.28) | 6.63 (12.13) | 14.24 (27.02) | 3.84 (8.31) | 0.03 (0.04) |
| Stenosis severity grade                           |         |       |       |       |        |       |        |       |
| High grade vs. low grade                           | -1.02 (0.65) | -57.22 (37.13) | -0.23 (0.29) | -17.04 (12.65) | -36.89 (28.15) | -9.15 (8.92) | 0.01 (0.05) |
| Subtotal stenosis vs. low grade                    | -0.87 (0.67) | -57.54 (38.29) | 0.13 (0.30) | -5.68 (13.04) | -11.17 (29.04) | 0.44 (8.92) | 0.06 (0.06) |
| Occlusion vs. low grade                            | -1.89 (0.70)* | -76.57 (40.22) | 0.15 (0.32) | -12.51 (13.70) | -26.47 (30.50) | -6.16 (9.36) | -0.01 (0.04) |

*P < 0.05.
**P < 0.01.

Bold values indicate statistically significant.

CRP indicates C-reactive protein; hs-CRP, hypersensitive C-reactive protein; IL-6, interleukin-6; NLR, neutrophil to lymphocyte ratio; PCT, procalcitonin; PLR, platelet to lymphocyte ratio; RDW, red blood cell distribution width; RIC, remote ischemic conditioning.
lower recurrence of stroke and decreased inflammatory and thrombophilia biomarkers (IL-6, CRP, PAI-1, PLT aggregation rate) after RIC intervention twice daily for half years.\textsuperscript{11} Taken all this together, it is indicated that older patients with cerebral arteriostenosis are more likely to have hyperinflammatory and hypercoagulation status, and maybe suggested to consider the long-term use of RIC as an adjunctive strategy.

Evaluations of single-time RIC effects only took place 5 minutes post-RIC, for several reasons. First, all participants served as their own controls. Second, based on previous animal and clinical studies which had shown instant proteomic changes following RIC, we aimed to evaluate probable changes as early as possible, and 5 minutes post-RIC was the earliest time point. Third, the acute effects of RIC stimulus on perfusion dynamics are mainly on cutaneous microcirculation, not general circulation.\textsuperscript{30} Fourth, the possibility of spontaneous changes in biomarkers due to flow-mediated dilation effects was relatively small. Finally, as all enrolled patients came from outpatient settings, long wait times are a big concern, and 5 minutes post-RIC allowed for a shorter timeframe.

There are several limitations of this study. First, as this was a prospective clinical study based on outpatient settings, the sample size was relatively small, and some patients did not have post-RIC data. However, this was the first study to evaluate the protective effects of RIC on cerebral arteriostenosis patients, so we limited our sample size to allow for close follow-up of these patients. Second, we did not have a sham control group because all participants served as their own controls and were studied twice: once at study enrollment and once right after completing their RIC treatment. Correction for multiple comparison was not conducted for this study. Finally, evaluating singular-time RIC effects could have been more precise by adding more sampling time, such as within 45 minutes, 24 hours and even 1 months, to more dynamically follow the post-RIC change of biomarkers.

**CONCLUSION**

RIC, as an extremely simple, noninvasive, and cost-effective method, showed seemingly coexisted anti-inflammatory and anti-coagulation effects of a single bout on patients with cerebral arteriostenosis. Older age was discovered as a negative predictor for multiple biomarkers, such as RDW, IL-6, CRP, and fibrinogen. Elderly people with cerebral arteriostenosis may benefit from using RIC as an adjunctive strategy. Whether there is a protective effect of RIC on cerebral arteriostenosis patients needs to be further studied in a larger sample size.

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