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Original Article

Plasma hydrogen sulfide as a promising predictor of collateral cerebral circulation in acute ischemic stroke

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Abstract: Objectives: To investigate the relationship between plasma hydrogen sulfide (H_2S) levels and collateral circulation in patients with acute ischemic stroke (AIS), and to explore the effects of vascular endothelial growth factor (VEGF), homocysteine (Hcy), folic acid (FA), vitamin B_{12} (VB_{12}), and vitamin B_6 (VB_6). Methods: A total of 68 AIS patients were enrolled and classified into two groups based on collateral vessel grading: the Good Collateral Circulation (GCC) group (n = 37) and the Poor Collateral Circulation (PCC) group (n = 31). Plasma levels of H_2S, VEGF, Hcy, FA, VB_{12}, and VB_6 were measured on the 2nd and 7th days after admission using microassays and ELISA. Results: Compared to the PCC group, patients in the GCC group had significantly lower National Institutes of Health Stroke Scale (NIHSS) and Modified Rankin Scale scores (mRS) scores at both admission and discharge (all $P < 0.05$). On both the 2nd and 7th days, plasma levels of H_2S, VEGF, FA, VB_{12}, and VB_6 were significantly higher in the GCC group, while Hcy levels were significantly lower (all $P < 0.05$). Within group comparisons between the two time points also showed significant changes (all $P < 0.001$). Correlation analysis revealed that plasma H_2S levels were positively correlated with collateral circulation, VEGF, FA, VB_{12}, and VB_6, and negatively correlated with Hcy levels (all $P < 0.001$). Plasma H_2S levels demonstrated high predictive value for collateral circulation (area under the curve, AUC = 0.943). An interaction between time and collateral circulation on H_2S levels was also observed. Conclusions: Plasma H_2S levels may serve as a valuable biomarker for predicting good collateral circulation in patients with acute ischemic stroke.

Keywords: Plasma hydrogen sulfide, collateral circulation, acute ischemic stroke, vascular endothelial growth factor, homocysteine

Introduction

Stroke is a common acute cerebrovascular disorder with significant sequelae, resulting from ischemia or hypoxia in brain tissue due to vascular obstruction or rupture. It primarily manifests in two forms: ischemic stroke (cerebral infarction) and hemorrhagic stroke (cerebral hemorrhage), with ischemic stroke being the more prevalent. Stroke is the second leading cause of death globally, with approximately 15 million people experiencing their first stroke each year [1]. According to the 2021 *Report on Stroke Prevention and Treatment in China*, the overall incidence of stroke in China continues to rise [2]. Although the rate of hemorrhagic stroke has declined, the incidence of ischemic stroke has increased in recent years, with a five year recurrence rate of 41% and a mortality rate of 16%. Stroke is now the leading cause of death and disability among adults in China [2]. Current treatments, such as intravenous thrombolysis and endovascular thrombectomy, aim to restore vascular patency, improving cerebral perfusion, and limit irreversible neurological damage in patients with acute ischemic stroke (AIS) [3, 4]. However, these therapies are constrained by narrow therapeutic time windows,

high costs, and associated complications, thus benefiting only a minority of patients.

Emerging research has shown that cerebral collateral circulation (CCC) is activated as a secondary compensatory mechanism following acute cerebral ischemia. CCC can provide supplementary perfusion to ischemic areas, making it a promising target for therapeutic intervention [5, 6]. CCC refers to alternative blood flow pathways that form when major cervical or intracranial vessels are stenosed or occluded. These collaterals bypass the obstructed vessels and deliver oxygen and nutrients to the ischemic penumbra, improving tissue viability. Studies have demonstrated a strong correlation between the presence of CCC and improved clinical outcomes after cerebral infarction [7-9]. Patients with well-developed CCC have been shown to exhibit lower NIH Stroke Scale (NIHSS) scores, smaller ischemic penumbra volumes, and reduced infarct core size compared to those with poor CCC [10]. Therefore, accurate assessment of CCC and its relationship to prognosis is of significant clinical importance.

Hydrogen sulfide (H_2S) is the third endogenous gaseous signaling molecule identified, primarily sunthesized in the central nervous system via the degradation of L-cysteine by cystathionine β-synthase (CBS) [11]. L-cysteine itself is a product of homocysteine (Hcy) metabolism. Hyperhomocysteinemia (HHcy), characterized by elevated plasma Hcy levels, is an established independent risk factor for cerebrovascular disease. HHcy may result from reduced CBS activity, genetic mutations in the 5,10-methylene tetrahydrofolate reductase (MTHFR) gene, and deficiencies in vitamin cofactors such as folic acid (FA), vitamin B_{12} (VB_{12}), and vitamin B_6 (VB_6). Additionally, reduced plasma H_2S concentrations may contribute to the development of HHcy in AIS [12, 13]. Previous studies have shown that serum H_2S levels peak within 24 hours of AIS onset, then decline between days 1 to 4 before partially rebounding on days 4 to 5, though still remaining below initial levels [14]. Moreover, serum H_2S levels have been inversely correlated with cerebral infarct volume. In an MCAO (middle cerebral artery occlusion) mouse model, Tao et al. [15] reported that low-dose sodium hydrosulfide (NaHS) administration reduced inflammatory responses and improved neurological function. Researchers Jiang et al. [16] found that NaHS injection in MCAO rats reduced infarct volume and the expression of apoptosis-related proteins, demonstrating a neuroprotective effect. Furthermore. Another study in hemorrhagic stroke models showed that NaHS promoted angiogenesis and improved neurological outcomes [17]. Despite these neuroprotective roles of H_2S, its involvement in the development of collateral circulation after cerebral infarction remains insufficiently understood. We hypothesize that H_2S may facilitate angiogenesis following cerebral ischemia and serve as an useful biomarker for evaluating CCC status.

Vascular endothelial growth factor (VEGF) plays a key role in neovascularization after cerebral ischemia [18]. Clinical studies have demonstrated elevated serum VEGF levels in stroke patients compared to healthy controls, with higher levels observed in those with good CCC compared to poor CCC [19]. In stroke model mice, intraventricular injection of VEGF increased microvessel density in the ischemic penumbra and reduced infarct size [20]. Zhang et al. [21] further reported that NaHS enhanced Akt phosphorylation, endothelial cell migration, and tube formation, while also upregulating VEGFR2 expression, thereby promoting angiogenesis and facilitating recovery from hypoxia-reoxygenation repair. These findings suggest that VEGF expression contributes to vascular remodeling and neuronal repair, potentially improving neurological outcomes. However, the relationship between plasma H_2S levels, VEGF concentrations, and the status of collateral circulation in AIS patients has not been fully elucidated. Therefore, this study aims to examine the correlation between peripheral blood H_2S levels and CCC in AIS, and to explore how VEGF, Hcy, FA, VB_6, and VB_{12} influence plasma H_2S levels. The goal is to identify reliable biomarkers for predicting CCC status and to provide novel insights into targeted therapeutic strategies.

Materials and methods

Subjects

A total of 68 inpatients diagnosed with cerebral infarction were retrospectively screened

between January 2023 and May 2024 at the Department of Neurology, Affiliated Hospital of Youjiang Medical University for Nationalities. Based on digital subtraction angiography (DSA) findings, patients were clssified into two groups: the Good Collateral Circulation (GCC) group (n = 37) and the Poor Collateral Circulation (PCC) group (n = 31). This study was approved by the Ethics Committee of the Affiliated Hospital of Youjiang Medical University for Nationalities (Approval Number: YYFY-LL-2023-039).

Inclusion criteria: (1) First episode of cerebral infarction within ≤ 3 days of symptom onset. Diagnosis was based on the Chinese Guidelines for Diagnosis and Treatment of Acute Ischemic Stroke [22], which defines acute onset with focal neurological deficits (e.g., unilateral facial or limb weakness/numbness, speech impairment) lasting over 24 hours, with cerebral infarction confirmed by cranial magnetic resonance diffusion-weighted imaging. Non-vascular etiologies were excluded. (2) DSA examination confirming middle cerebral artery stenosis or occlusion ≥ 70% on one side of the brain. (3) Classification into the large-artery atherosclerosis subtype based on the Trial of Org 10172 in Acute Stroke Treatment classification [23]. (4) Patients who exceeded the time window for intravenous thrombolysis and arterial thrombectomy at admission and did not receive endovascular treatment. (5) Age between 18 and 80 years. (6) Complete clinical data.

Exclusion criteria: (1) History of cerebral infarction, cerebral hemorrhage, craniocerebral trauma, brain tumor, or similar conditions. (2) Cerebral infarction due to cardiogenic embolism, intracranial infection, vasculitis, subarachnoid hemorrhage, intracranial tumor, or blood disorders. (3) Serious concurrent diseases such as heart, lung, or liver diseases, or complications like renal insufficiency, severe infections, or tumors. (4) Failure to cooperate with blood reexamination, cranial MRI, or DSA examination. (5) Incomplete clinical data or refusal to participate.

Assessment of collateral cerebral circulation and grouping

On the day after admission, patients underwent DSA examination after providing informed consent. The degree of cerebral artery stenosis was calculated using the formula: intracranial artery stenosis rate = (1- the diameter of the narrowest intracranial artery/the diameter of the normal part) × 100% [24].

CCC was evaluated by two interventional physicians using the collateral vessel grading system from the American Society of Interventional and Therapeutic Neuroradiology/Society of Interventional Radiology [25]. The CCC state was categorized as “good” (grades 0-1) or “poor” (grades 2-4), and patients were assigned to the Good Collateral Circulation (GCC) group (n = 37) and the Poor Collateral Circulation (PCC) group (n = 31) accordingly. A typical DSA image of patients in each group is shown in **Figure 1**.

Treatment plan

All patients with cerebral infarction were treated with aspirin and/or clopidogrel hydrogen chloride, atorvastatin, butylphthalide, urinary kallikreinogenase, idebenone, and other drugs according to the Chinese Guidelines for Diagnosis and Treatment of Acute Ischemic Stroke. Patients with hypertension and diabetes received appropriate antihypertensive and hypoglycemic treatments. Edaravone, FA, VB_6, and VB_{12} were not administered to any patient before or during hospital treatment.

Clinical data collection

Age, gender, blood glucose, blood pressure, medical history (diabetes, hypertension, hyperlipidemia, stroke, coronary heart disease, etc.), smoking history, drinking history, and other relevant information were collected for all included subjects. The National Institutes of Health Stroke Scale (NIHSS) and Modified Rankin Scale (mRS) scores were recorded by the attending physician at the time of admission, taking the patient’s condition into consideration, and the prognosis was assessed by the neurologist using the NIHSS and mRS scores again at the time of discharge.

Sample collection

Fasting venous blood (3 mL) was collected from patients with cerebral infarction on the 2nd and 7th days of admission, using an EDTA anticoagulation tube. After collection, the blood was gently mixed by inversion, left to stand for 20 minutes, and then centrifuged at 3000 rpm for 20

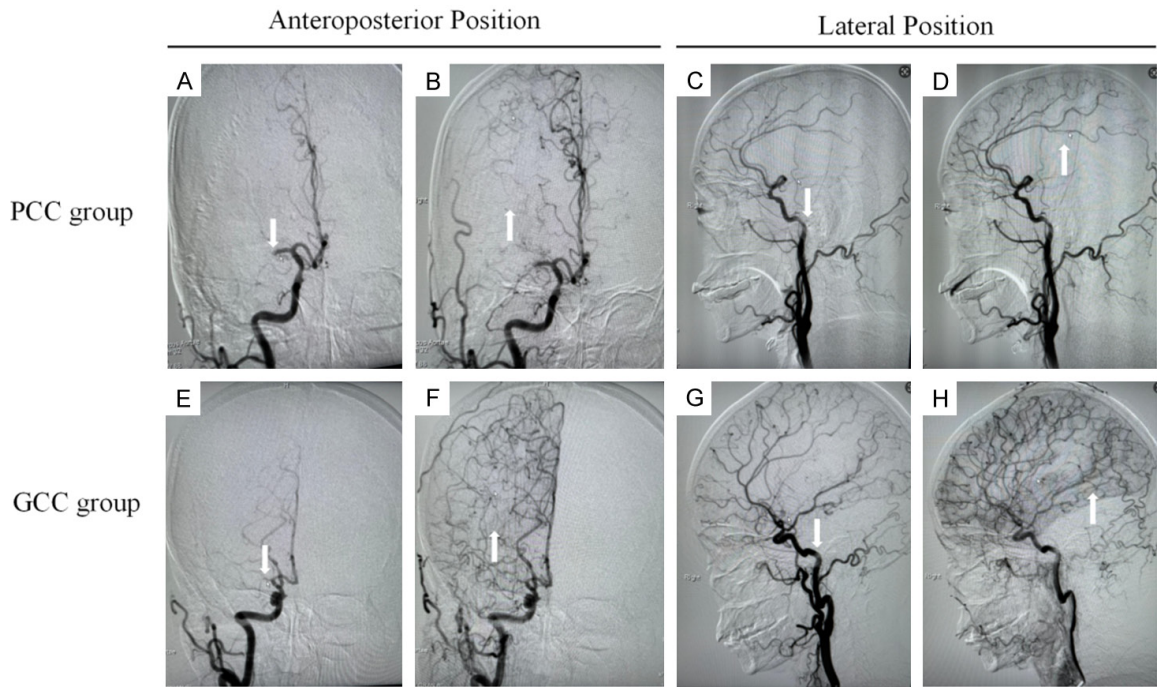


Figure 1. DSA results of different collateral circulation states. Panels (A-D) show the DSA results on the day after admission for a patient in the PCC group. Panels (A and C): Right middle cerebral artery occlusion (anteroposterior and lateral views). Panels (B and D): The right anterior cerebral artery leptomeningeal vessels compensating for the pericortical blood supply to the ipsilateral middle cerebral artery, with sparse and slow blood flow, and a collateral circulation score of 1 (anteroposterior and lateral views). Panels (E-H) show the DSA results on the day after admission for a patient in the GCC group. Panels (E and G): Right middle cerebral artery occlusion (anteroposterior and lateral views). Panels (F and H): Formation of leptomeningeal vessels and capillary networks from the right external carotid artery and right anterior cerebral artery, compensating for the blood supply to the entire region of the ipsilateral middle cerebral artery, with a collateral circulation score of 4 (anteroposterior and lateral views). DSA, digital subtraction angiography; PCC, poor collateral circulation; GCC, good collateral circulation.

minutes. The supernatant was transferred into a sterile high-pressure 1.5 mL EP tube for subsequent analysis. The remaining samples were aliquoted and stored at -80°C to avoid repeated freezing and thawing.

Detection of plasma H_2S level by microassay

A 150 μL plasma sample was added to the sample tube, and a blank tube was prepared. Samples were processed according to the instructions provided with the H_2S kit (Enzyme-linked Biological, ml076943). OD values for each well were measured using an enzyme-labeled instrument, and ΔA values (A determination - A blank) for each well were calculated. H_2S concentration was then calculated using the following formula:

$$H_2S\ (nmol/mL) = \frac{\triangle A}{0.0022} \times V_{total} \div V_{sample} = 681.8 \times \Delta A$$

V_{total}: total reaction volume; V_{sample}: volume of sample in reaction.

Detection of plasma VEGF, VB_6, VB_{12}, FA and HCY levels by ELISA

A 50 μL plasma sample was added to the reaction well, with blank wells, control group wells, and standard curve wells prepared. The assays for VEGF (Quanzhou Ruixin Biotechnology Co., Ltd., RX105003H), VB_6 (Quanzhou Ruixin Biotechnology Co., Ltd., RXJ105135H), VB_{12} (Quanzhou Ruixin Biotechnology Co., Ltd., RXJ105137H), FA (Quanzhou Ruixin Biotechnology Co., Ltd., RXJ106799H), and Hcy (Quanzhou Ruixin Biotechnology Co., Ltd., RXJ105166H) were conducted according to the manufacturers' instructions. Enzyme-labeled antibodies were added for incubation. After incubation, the wells were washed with PBS, followed by the addition of substrate

Table 1. Comparison of clinical data (n (%), $\bar{X}\pm s$, M (P_{25}, P_{75}))

	GCC group (n = 37)	PCC group (n = 31)	$t/Z/\chi^2$	*P*
Gender (Male/Female)	25/12	23/8	0.466	0.495
Age (years)	56 (49.00, 66.00)	63 (55.00, 70.00)	1.782	0.075
Diabetes history	9 (23.1%)	4 (12.9%)	0.605	0.432
Hypertension history	32 (82.1%)	17 (54.8%)	6.090	0.014
Smoking history	21 (53.8%)	9 (29.0%)	4.342	0.037
Alcohol history	21 (53.8%)	10 (32.3%)	3.262	0.071
mRS score at admission	2.0 (2.0, 3.0)	3.0 (2.0, 4.0)	3.841	< 0.001
mRS score at discharge	1.0 (1.0, 2.0)	2.0 (1.0, 2.0)	3.701	< 0.001
NIHSS score at admission	10.0 (8.0, 14.0)	16.0 (12.0, 20.0)	4.454	< 0.001
NIHSS score at discharge	6.36 ± 2.87	10.26 ± 3.45	-5.159	< 0.001

Note: GCC, Good Collateral Circulation; PCC, Poor Collateral Circulation; mRS, Modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale.

developing solution for color reaction. The reaction was stopped by adding the corresponding stop solution, and optical density was measured using a microplate reader. The concentrations of VEGF, VB_6, VB_{12}, FA, and Hcy in each plasma sample were determined from the standard curve values.

Statistical analysis

Data were analyzed and visualized using GraphPad Prism 9.0 software. Normality testing was performed on all measurement data. Data conforming to a normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm sd$). An independent sample t-test was used for comparisons between two groups, while a paired sample t-test was used for comparisons at different time points within a group. Data that were not normally distributed were expressed as M (P_{25}, P_{75}), and the Mann-Whitney U test was used for comparisons between two samples. Count data were presented as frequency (rate) and analyzed using the χ^2 test. Spearman rank correlation was used to assess the correlation between H_2S concentration and collateral circulation state, as well as between H_2S and VEGF, Hcy, FA, VB_6, and VB_{12}. Differences were considered statistically significant at $P < 0.05$.

Results

Comparison of clinical data

No significant differences were observed between the groups regarding age, gender, diabetes, or drinking history ($P > 0.05$). The NIHSS and mRS scores on admission and discharge were significantly higher in the PCC group compared to the GCC group (all $P < 0.001$), as shown in **Table 1**.

Comparison of plasma H_2S levels

To evaluate the plasma H_2S levels in patients under different CCC states, H_2S was measured using a microassay method. Results showed that plasma H_2S levels in the GCC group were significantly higher than those in the PCC group on both the 2nd and 7th days of admission (t = 13.875 and 8.776, both $P < 0.001$). Additionally, plasma H_2S levels on the 7th day were significantly higher than on the 2nd day for both groups (t = 8.271 and 6.817, both $P < 0.001$). See **Figure 2**.

Comparison of plasma VEGF levels

Plasma VEGF levels were measured by ELISA to assess the VEGF concentrations under different collateral circulation states. The results indicated that plasma VEGF levels were significantly higher in the GCC group compared to the PCC group on both the 2nd and 7th days of admission (t = 43.894 and 64.245, both $P < 0.001$). The plasma VEGF levels in both groups were higher on the 7th day than on the 2nd day (t = 18.021 and 38.339, both $P < 0.001$). See **Figure 3**.

Comparison of plasma HCY, FA, VB_6 and VB_{12} levels

Hcy generates L-cysteine and H_2S through CBS, and its level is influenced by cofactors FA, VB_6,

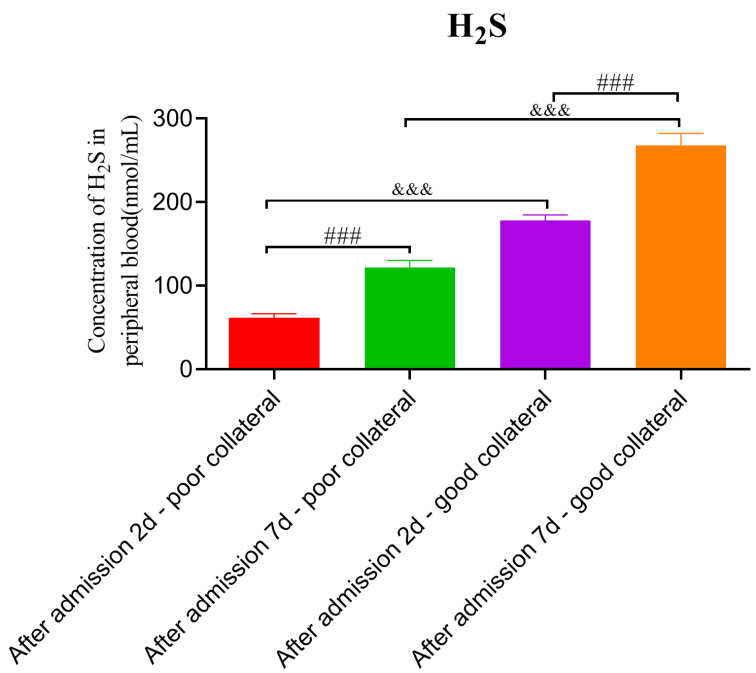


Figure 2. Levels of H_2S in peripheral blood in different collateral circulation. Note: Paired *t* test $^{###}P < 0.001$; independent *t* test, $^{&&&}P < 0.001$. Plasma hydrogen sulfide (H_2S).

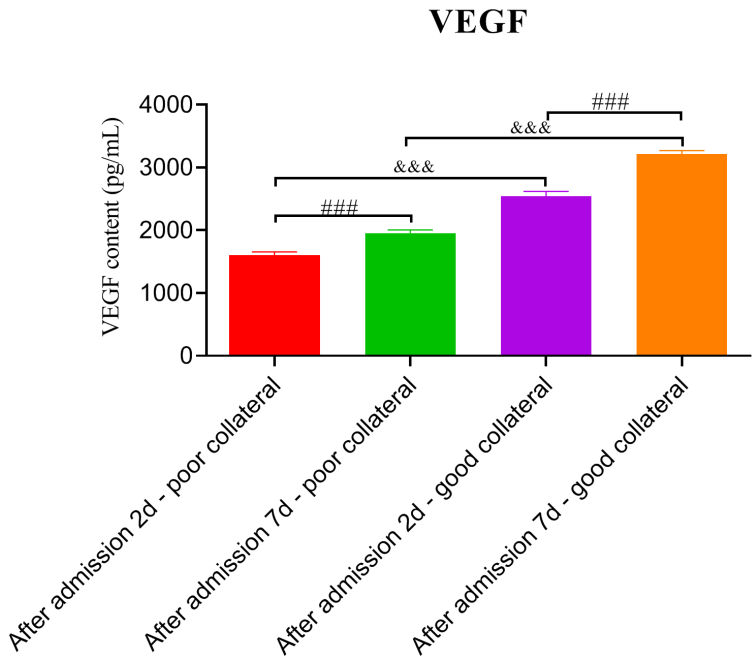


Figure 3. Peripheral blood VEGF levels in different collateral circulation. Note: Paired *t* test $^{###}P < 0.001$; independent *t* test $^{&&&}P < 0.001$. VEGF, vascular endothelial growth factor.

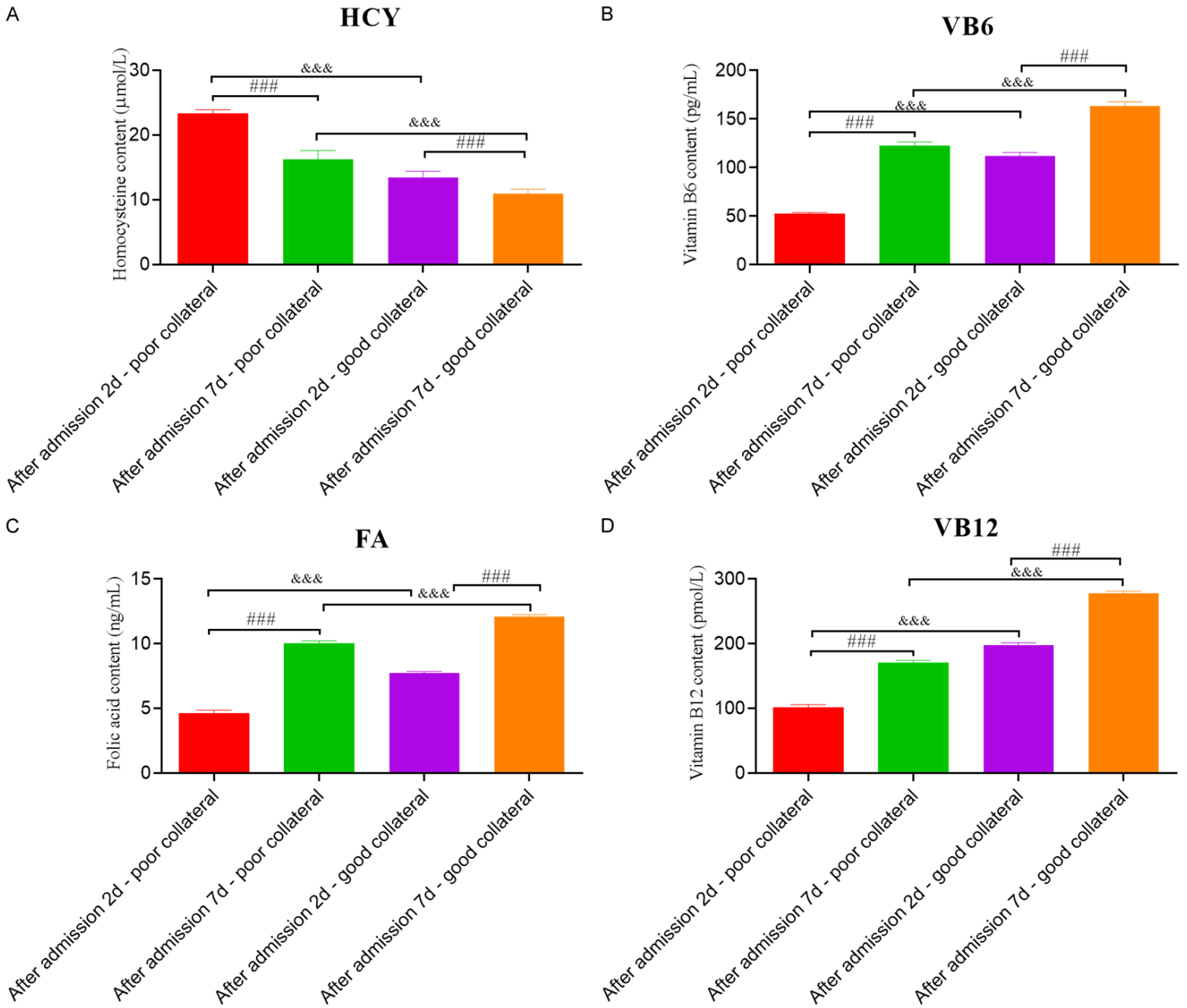
and VB_{12}. Plasma levels of Hcy, FA, VB_6, and VB_{12} were measured by ELISA to explore their relationship in the two groups. The results showed that plasma Hcy levels in the PCC group were significantly higher than in the GCC group on both the 2nd and 7th days of admission (t = 16.637 and 13.264, both $P < 0.001$). Plasma Hcy levels on the 7th day in both groups were significantly lower than on the 2nd day (t = 6.371 and 13.090, both $P < 0.001$). Regarding plasma VB_6, VB_{12}, and FA, the PCC group had significantly lower levels than the GCC group on both the 2nd and 7th days (t = 18.626, 7.306 for VB_6; t = 19.400, 19.630 for VB_{12}; t = 13.464, 7.791 for FA; all $P < 0.001$). On the 7th day, plasma VB_6, VB_{12}, and FA levels were higher than on the 2nd day in both groups (t = 18.969, 11.547 for VB_6; t = 11.674, 19.630 for VB_{12}; t = 21.048, 17.977 for FA; all $P < 0.001$). See **Figure 4**.

Correlation analysis of H_2S and collateral circulation state

To explore the correlation between plasma H_2S levels and collateral circulation status, patients' collateral circulation scores were assigned, with collateral circulation dysfunction = 1 and GCC = 2. Bivariate Spearman's correlation analysis showed that plasma H_2S levels were positively correlated with collateral circulation status, with a correlation coefficient of 0.855 ($P < 0.001$), as shown in **Table 2**.

Correlation analysis between H_2S and levels of VEGF, Hcy, FA, VB_6 and VB_{12}

To explore the correlation between plasma H_2S levels and VEGF, Hcy, FA, VB_6, and VB_{12} levels in the two patient groups, a bivariate Spearman's correlation analysis was per-


A
HCY
Homocysteine content (μmol/L)
0
10
20
30
&&&
###
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After admission 2d - poor collateral
After admission 7d - poor collateral
After admission 2d - good collateral
After admission 7d - good collateral
B
VB6
Vitamin B6 content (pg/mL)
0
50
100
150
200
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After admission 2d - poor collateral
After admission 7d - poor collateral
After admission 2d - good collateral
After admission 7d - good collateral
C
FA
Folic acid content (ng/mL)
0
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After admission 2d - poor collateral
After admission 7d - poor collateral
After admission 2d - good collateral
After admission 7d - good collateral
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VB12
Vitamin B12 content (pmol/L)
0
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After admission 2d - poor collateral
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After admission 2d - good collateral
After admission 7d - good collateral

Figure 4. Levels of Hcy, FA, VB_6 and VB_{12} in peripheral blood in different collateral circulation. Note: Paired *t* test $^{###}P < 0.001$; independent *t* test $^{\&\&\&}P < 0.001$. Hcy, homocysteine; VB_6, vitamin B_6; FA, folic acid; VB_{12}, vitamin B_{12}.

Table 2. Correlation analysis between H_2S and collateral circulation score

	r	*P*
collateral circulation & H_2S	0.855	< 0.001

Note: valuation of collateral circulation: collateral circulation dysfunction = 1; GCC =2, PCC group n = 31, GCC group n = 37.

Table 3. Correlation analysis between H_2S and each index

	r	*P*
VEGF	0.696	< 0.001
Hcy	-0.664	< 0.001
FA	0.759	< 0.001
VB_{12}	0.610	< 0.001
VB_6	0.543	< 0.001

Note: VEGF, vascular endothelial growth factor; Hcy, homocysteine; FA, folic acid; VB_{12}, vitamin B_{12}; VB_6, vitamin B_6.

formed. The results showed that plasma H_2S levels were positively correlated with VEGF, FA, VB_6, and VB_{12}, with correlation coefficients of 0.696, 0.759, 0.610, and 0.543, respectively (all $P < 0.001$). Conversely, plasma H_2S levels were negatively correlated with Hcy, with a correlation coefficient of -0.664, which was also statistically significant ($P < 0.001$). Detailed results are presented in **Table 3**.

Predictive value of H_2S for collateral circulation

Receiver Operating Characteristic (ROC) curve analysis revealed that the Area Under the Curve (AUC) for plasma H_2S level as a predictor of collateral circulation was 0.943 (95% Confidence Interval [CI]: 0.907-0.978), with a diagnostic sensitivity of 60.81% and specificity of 98.39% ($P < 0.001$, **Figure 5**).

Interaction of time and collateral circulation on H_2S change

Interactive analysis demonstrated that after adjusting for related variables (including VEGF, Hcy, FA, VB_6, and VB_{12} levels), the change in H_2S levels between the two groups was significantly different (all $P < 0.001$, **Figure 6**).

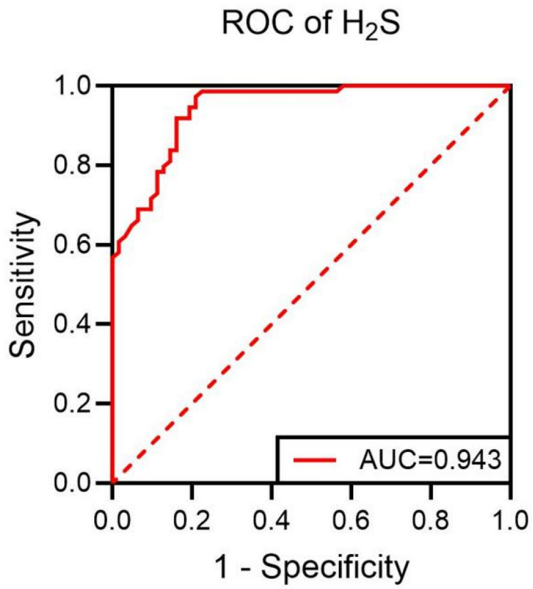

Figure 5. ROC curve of H_2S expression on collateral circulation. ROC, receiver operator characteristic curve; AUC, Area Under The Curve. Plasma hydrogen sulfide (H_2S).

Discussion

Cerebral infarction is a prevalent cerebrovascular condition characterized by high rates of mortality and recurrence, posing a substantial economic burden on patients and families and increasing psychological stress [26]. The formation of collateral circulation plays a pivotal role in both the treatment and prognosis of cerebral infarction. Upon onset, collateral vessels facilitate blood flow to ischemic regions, thereby enhancing vascular recanalization and maintaining perfusion in the ischemic penumbra. This can ultimately help reduce infarct size [27]. Good collateral circulation (GCC) is also a key factor in selecting candidates for endovascular intervention [28]. Notably, the recanalization rate in patients with GCC after thrombolysis reaches 61.80%, compared to only 28.10% in those with poor collateral circulation (PCC) [29], highlighting the critical value of GCC in endovascular treatment strategies.

In this study, we used the ASITN/SIR collateral grading system based on DSA imaging to assess collateral circulation. This system is

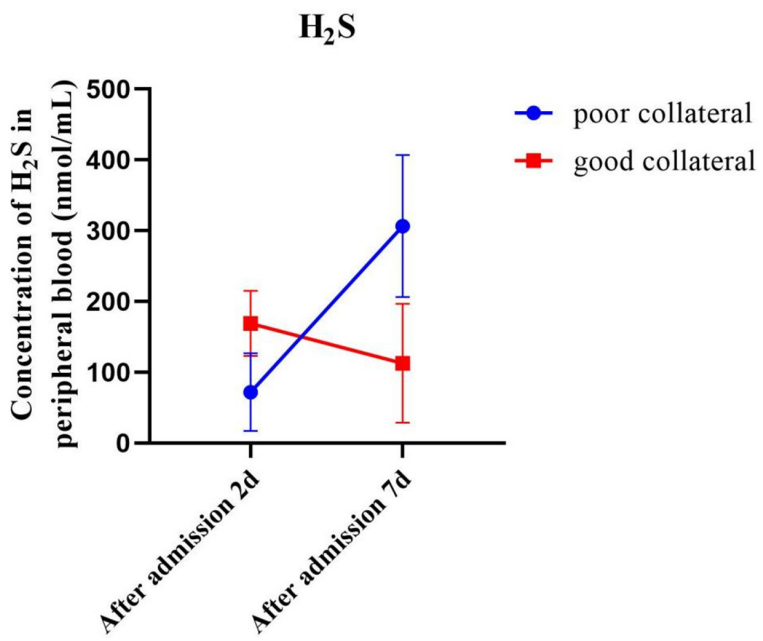

Figure 6. Interaction of time and collateral circulation on H_2S change. Plasma hydrogen sulfide (H_2S).

widely utilized due to its reproducibility and diagnostic consistency [30, 31]. A prospective study from the Interventional Management of Stroke III trial showed that patients with ASITN/SIR grades 3 or 4 had significantly better rates of recanalization, reperfusion, and functional recovery [32]. Collateral circulation development is influenced by age, genetics, and various cerebrovascular risk factors or biomarkers [33]. Homocysteine (Hcy) has been identified as a key risk factor; vitamins B_6, B_{12}, and folic acid (FA) are integral to Hcy metabolism [12]. Additionally, vascular endothelial growth factor (VEGF) is a well-established regulator of collateral formation in ischemic stroke [34]. Thus, we explored peripheral blood levels of H_2S, VEGF, VB_6, VB_{12}, Hcy, and FA in cerebral infarction patients to assess their associations with collateral circulation.

Hydrogen sulfide (H_2S), a gaseous signaling molecule, has attracted growing attention in stroke research. Inflammatory responses following cerebral infarction may impact cerebral hemodynamics and collateral vessel formation. Accumulating evidence indicates that H_2S exerts anti-inflammatory, antioxidant, and neuroprotective effects in neurological disorders [35]. It has been shown to protect against cerebral ischemia-reperfusion injury, potentially by suppressing excessive autophagy activation [36]. Thus, increased H_2S levels may improve cerebral blood flow, enhance microcirculation, and facilitate collateral development. Plasma H_2S levels have been implicated in stroke pathophysiology [14]; in a study of 71 elderly stroke patients, plasma H_2S levels initially declined and later increased as the disease progressed, with a negative correlation observed between H_2S levels and infarct size. Our results were consistent with this trend-plasma H_2S levels on days 2 and 7 post-admission were significantly higher in the GCC group versus the PCC group, and levels rose from day 2 to day 7 in both groups. Additionally, NIHSS and mRS scores at admission and discharge were significantly lower in the GCC group. These findings suggest that cerebral ischemia may stimulate H_2S production, offering neuroprotection. Plasma H_2S may thus serve as a potential biomarker for predicting GCC. One study [37] indicated that the neuroprotective effects of H_2S are dose-dependent: low concentrations confer benefits, while high concentrations may cause neurotoxicity. Our data suggest that levels between 61-268 μmol/L are within the protective range and may facilitate collateral formation, potentially through H_2S-mediated VEGF upregulation.

VEGF is a key factor in angiogenesis and collateral vessel formation after stroke [19, 38]. It promotes endothelial cell proliferation and migration, enhancing vascular regeneration and perfusion of ischemic areas [39]. Animal studies revealed that VEGF mRNA peaks between days 1-3 following hypoxia and declines after day 7 [40], suggesting a time-sensitive regulatory role in ischemia. In this study, VEGF treatment of cortical neurons increased Bcl-2 while reducing Bax and TREK protein levels, suggesting a neuroprotective mechanism via TWIK channels. Li et al. [41] reported that VEGF levels rise early after AIS onset and peak at 506.68 ng/L between days 3-7, correlating with angiogenesis and collateral formation. In

our study, VEGF levels were significantly higher in the GCC group on both day 2 and day 7, with levels rising over time in both groups. These results affirm the role of VEGF in collateral development and suggest that elevated VEGF may serve as a marker of GCC.

Our findings further suggest a synergistic relationship between H_2S and VEGF in collateral circulation. H_2S modulates angiogenesis, endothelial proliferation, and vascular tone, partly by stimulating VEGFR2 signaling and reducing intracellular calcium [42]. H_2S may directly act on VEGF receptors by disrupting disulfide bonds (Cys1045-Cys1024) in VEGFR2, enhancing endothelial activity [43]. Spearman correlation analysis showed a positive correlation between H_2S and VEGF levels, supporting a regulatory interaction that may promote vascular remodeling and collateral development.

Hcy impairs endothelial function by generating reactive oxygen species (ROS), promoting apoptosis, and inhibiting VEGF expression, thereby hindering collateral formation [44]. Elevated Hcy increases platelet aggregation and blood viscosity, further limiting perfusion [45]. Our study found that Hcy levels were significantly higher in the PCC group on both day 2 and day 7, consistent with these mechanisms.

Previous studies have demonstrated a negative correlation between H_2S and Hcy levels in stroke patients [12], a finding replicated in our study. This suggests that H_2S may attenuate Hcy toxicity, promoting collateral formation. Moreover, Hcy is metabolized via remethylation and transsulfuration pathways, requiring VB_{12}, FA, and VB_6. Deficiencies in these vitamins result in elevated Hcy levels, a known independent risk factor for stroke [46]. Studies have shown that low FA and VB_{12} increase stroke risk [47], and lower levels of these vitamins are observed in stroke patients with cognitive impairment [48]. Clinical intervention with FA and VB_6 reduces Hcy and improves neurological scores in AIS [49]. In our study, levels of VB_6, VB_{12}, and FA were significantly lower in the PCC group and positively correlated with H_2S, while Hcy levels showed an inverse trend. These results suggest that low vitamin levels contribute to PCC, and the H_2S-Hcy-vitamin axis may influence collateral status.

This study has limitations. Its retrospective, single-center design and small sample size may affect generalizability and introduce bias. Furthermore, some relevant markers could not be evaluated due to the retrospective nature. Future multicenter, prospective studies are needed to confirm our findings and elucidate the underlying mechanisms.

In conclusion, plasma H_2S levels are positively associated with GCC in cerebral infarction and may serve as a predictive biomarker. H_2S correlates positively with VEGF, FA, VB_6, and VB_{12} levels, and negatively with Hcy, suggesting its pivotal role in regulating collateral circulation and neurological outcomes in stroke.

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Disclosure of conflict of interest

None.

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