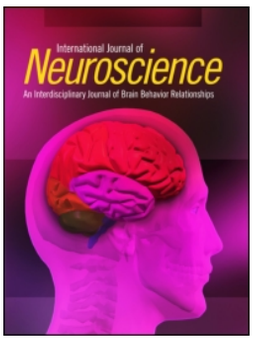

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ORIGINAL ARTICLE

Effects of hyperhomocysteinemia on ischemic cerebral small vessel disease and analysis of inflammatory mechanisms

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ABSTRACT

Purpose: Hyperhomocysteinemia is closely related to, but is not a confirmed risk factor of, cerebral small vessel disease (CSVD). This study aimed to determine whether hyperhomo-cysteinemia is correlated significantly with CSVD.

Materials and methods: This cross-sectional study compared the homocysteine (Hcy) levels of patients with and without CSVD. High-sensitivity C-reactive protein (hs-CRP) levels were compared according to white matter lesion (WML) severity, which was classified using the Fazekas system. Risk factors for ischemic CSVD were analyzed through multivariate unconditional logistic regression analysis.

Results: Hcy levels were significantly higher in patients with lacunar infarction (LI) than in controls (p=.0438), in patients with Fazekas 2–3 than in patients with Fazekas 0–1 WMLs (p=.0192), in patients with Fazekas 4–6 than in patients with Fazekas 2–3 WMLs (p=.0207), and in patients with LI than in patients without LI (p=.0043). hs-CRP levels were significantly higher in patients with LI than in patients without LI (p=.0068) and in patients with Fazekas 4–6 than in patients with Fazekas 0–1 WMLs (p=.0031). Three multivariate unconditional logistic regression analyses showed that hyperhomocysteinemia is a risk factor for LI (p=.006; odds ratio [OR], 27.668), severe WML (p=.028; OR, 1.984), and high hs-CRP level (p=.016; OR, 3.956).

Conclusions: The assessment of Hcy levels is important for ischemic CSVD. Hyperhomocysteinemia is a risk factor for LI and severe WML. Further, hyperhomocysteinemia is associated with high hs-CRP levels, and this may involve an inflammatory mechanism; however, further studies are needed in this regard.

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Hyperhomocysteinemia; Homocysteine; Cerebral small vessel diseases; white matter lesions; C-reactive protein; lacunar infarcts

Introduction

The very high disability rate after a person experiences stroke can greatly influence their quality of life. Stroke was the third-highest condition that resulted in disability-adjusted life-year loss in the United States in 2010 [1] and generally poses a heavy burden on society and family. Ischemic strokes account for 67.3%−80.5% of all strokes [2]. Lacunar infarction (LI) is the most common subtype of first-ever ischemic stroke (54.1%) [3]. It is usually caused by intracranial atheromatous branch disease or occlusion of a single penetrating artery by microatheromas or lipohyalinosis [4]. Although chronic cerebral hypoperfusion does not cause LI, it often causes demyelination of the white matter of the brain. Imaging manifestations of demyelinating encephalopathy are mainly white matter lesions (WMLs), with ischemic WML being the most common type. Cerebral small vessel disease (CSVD) is usually defined as a syndrome that involves clinical, cognitive, imaging, and pathological manifestations caused by various pathological changes in the small vessels [5]. The CSVD manifests mainly as LI, WMLs, enlarged perivascular space, and cerebral microhemorrhage on magnetic resonance imaging (MRI), with LI and WMLs being the most common manifestations that collectively represent ischemic CSVD. An epidemiological survey of CSVD in Asian countries revealed significantly higher incidence of CSVD in elderly people, and the incidence increases with age [6]. WMLs gradually lead to intellectual impairment, gait disorders, bladder function disruption, mood disorders, and other disabilities. Prevention of ischemic CSVD is important for reducing the burden of the disease on

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Table 1. Severity and score of the paraventricular and deep WMLs: Fazekas classification.

Paraventricular WMLs	Deep WMLs	Score
No WMLs (Figure 1A)	No deep WMLs (Figure 1B)	0
Cap-shaped high signal in the occipital horn or frontal horn or thinner ring around at the edge of lateral ventricle (Figure 1C)	Spotted or patchy deep WMLs (Figure 1D)	1
Periventricular halo WMLs with smooth edge (Figure 1E)	Widespread patchy WMLs and early integration trend (Figure 1F)	2
Rough and thicker periventricular lesions with irregular margins protruding to the cerebral cortex (Figure 1G)	Extensive integration of deep WMLs (Figure 1H)	3

WMLs, white matter lesions.

the family and society. High levels of homocysteine (Hcy) increase the risk of having an ischemic stroke [7]. A potential mechanism underlying the induction of ischemic CSVD by hyperhomocysteinemia may involve inflammation. Inflammatory cytokine levels are elevated in individuals with hyperhomocysteinemia [8]. Further, inflammation plays an important role in the development of ischemic stroke and may be the underlying cause of stroke [9]. High-sensitivity C-reactive protein (hs-CRP), which plays an important role in the atherosclerotic plaque formation, is a commonly used inflammatory marker [10]. Elevated hs-CRP levels can damage vascular endothelial cells through activation of inflammatory reactions and induction of oxidative stress. However, the exact mechanism underlying the induction of inflammation by hyperhomocysteinemia is currently unknown. We aimed to investigate whether hyperhomocysteinemia is a risk factor for CSVD and is correlated with hs-CRP levels.

Materials and methods

Overall, 256 consecutive CSVD patients (male, $n = 151$; female, $n = 105$; age: mean ± standard deviation, 66.12 ± 13.29 years; range, 26–90 years) admitted to the Department of Neurology at our hospital between January 2017 and February 2018 were enrolled. CSVD patients included 103 LI patients and 153 WML patients. The LI control group ($n = 134$) included 43 inpatients with benign paroxysmal positional vertigo, 20 with Ménière disease, 37 with transient ischemic attacks, 21 with facial neuritis, and 13 with herpes zoster. The serum creatinine levels in the LI and non-LI groups were 65.00 ± 14.67 and 62.74 ± 14.64 mmol/L, respectively ($p < .05$). A total of 82 in-patients had severe WML, and 177 had non-severe WML. The non-severe WML group included 50 patients with transient ischemic attack, 25 with facial neuritis, 88 with peripheral vertigo, and 14 with herpes zoster. The serum creatinine levels in the severe and non-severe WML groups were 68.64 ± 12.27 and 64.74 ± 10.55 mmol/L, respectively ($p < .05$). The classification of ischemic stroke was performed according to the Trial of Org 10172 in Acute Stroke Treatment classification standards [11]. Overall, 1,377 patients who underwent physical examination in our hospital from January 2017 to February 2018 were selected as the control group, and their fasting venous blood Hcy levels were examined.

Patients with ischemic WML were aged from 31–90 years old and had no serious infection at the time of the study, no history of severe craniocerebral trauma, malignant tumors, or organ failure (e.g. heart, liver, kidney, lung, or other), no serious disease leading to a terminal state, no currently active immune disease, and no WMLs caused by poisoning, hereditary degeneration, metabolic diseases, or hydrocephalus were included. Patients with dysarthria-clumsy hand syndrome (DCHS) met the following criteria [12]: dysarthria without dysphasia; unilateral "central" facial weakness with ipsilateral clumsiness appearing as a cerebellar-type ataxia (dysmetria, dysrhythmia, dysdiadochokinesia, gait ataxia) or mild or no weakness; and no sensory symptoms or signs. The other lacunar syndrome group included all patients with LI, with the exception of DCHS. This study was approved by the Ethics Review Committee of the 3rd Affiliated Hospital of Shenzhen University (approval no. 2018LHYYSJNL-003-01), and written informed consent was obtained from all patients (or their parents or guardians).

All hospitalized patients underwent MRI. The Siemens 1.5-T MRI system (Siemens, Magnetic Resonance Co., Ltd., Munich, Germany) was used for brain MRI, including T1- and T2-weighted imaging (T1-WI and T2-WI, respectively), fluid-attenuated inversion recovery (FLAIR) sequences, and magnetic resonance angiography (MRA). An interval of 1.5 mm and 5-mm slice thickness were applied. The paraventricular and deep WMLs were scored based on the Fazekas classification method (0–6 points, see Table 1), and the total score was the sum of the paraventricular and deep WML scores (typical WMLs of different degree are shown in Figure 1). Cervical arteries were examined using an iE Elite ultrasonic diagnostic instrument

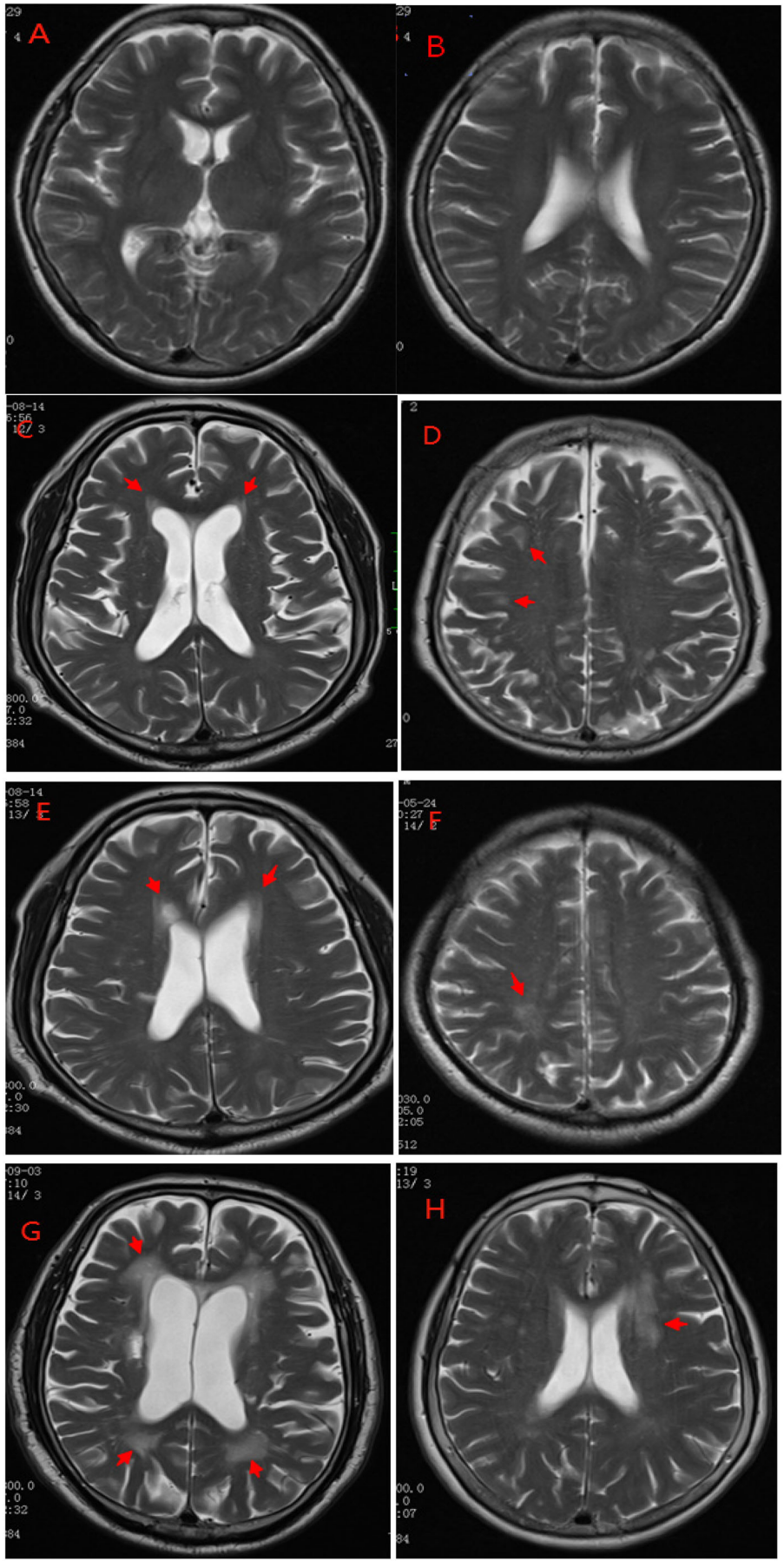

Figure 1. (A) No white matter lesion (WML) near the lateral ventricle; (B) no deep WML; (C) cap-shaped high signal in the occipital horn or frontal horn or thinner ring around at the edge of the lateral ventricle (arrow); (D) spotted or patchy deep WMLs (arrows); (E) periventricular halo WMLs with smooth edge (arrows); (F) widespread patchy WMLs and early integration trend (arrow); (G) rough and thicker periventricular lesions with irregular margins protruding to the cerebral cortex (arrows); (H) extensive integration of deep WMLs (arrow).

Table 2. Comparison of clinic character between patients with DCHS and patients with other lacunar syndromes.

	DCHS ($n = 8$)	Other LIs ($n = 95$)	P-value
Sex, male	5 (62.5)	57 (60)	NS
Age (years)	66.73 ± 13.51	66.78 ± 12.73	NS
Pure motor hemiparesis	0 (0)	27 (28.4)	.079
Pure sensory	0 (0)	26 (27.4)	.089
Sensorimotor syndrome	1 (12.5)	10 (10.5)	NS
Ataxia-hemiparesis	1 (14.3)	6 (6.3)	NS
Dysarthria	8 (100)	7 (7.4)	<.001
Dysarthria-clumsy hand	8 (100)	0 (0)	<.001
Atypical lacunar syndromes	0	19 (20)	NS

Values are presented as n (%) or mean ± standard deviation.
DCHS, dysarthria-clumsy hand syndrome; LI, lacunar infarction; NS, not significant.

(Philips, Amsterdam, the Netherlands), with a probe frequency of 10–15 MHz. The intima media thickness (IMT) of the carotid artery was recorded and defined as the vertical distance between the upper edge of the intima and the lower edge of the media. WMLs were categorized as mild, moderate, or severe according to the Fazekas scores: 0, normal; 1, mild; ≥2, moderate; ≥3, severe. A single neurologist scored all WMLs.

All included participants ate a low-protein diet for 1 day before blood collection. Blood samples were collected from elbow veins 12 h after fasting. We used the Beckman Au5821 automatic biochemical analyzer (Beckman Coulter Inc., California,The USA) to determine the Hcy levels using the rate method and a kit from Qiangsheng Biotechnology Co., Ltd. (Jinhua City, China). The normal reference range of Hcy was 5–15 mmol/L. Hcy levels >15 mmol/L were defined as hyperhomocysteinemia. High hs-CRP level was defined as hs-CRP >8 mg/L.

The diagnosis of hypertension was based on the 2010 China Hypertension Guideline [13]. Type 2 diabetes was diagnosed according to the China Prevention and Cure Guidelines (2017) [14]. The diagnosis of hyperlipidemia was based on the Profile and Interpretation of Prevention and Cure Guidelines (2007) [15]. Hyperuricemia was diagnosed according to consensus of the Internal Medicine Branch of Cardiovascular Diseases and the Council of Evidence-based Medicine of the Chinese Medical Association [16]. The diagnosis of ischemic stroke was based on the China Prevention and Cure Guideline to Cerebral Vascular Disease [17]. LI is defined as a small infarct (2–20 mm) of the basal ganglia, thalamus, or brainstem and appears as a cerebrospinal fluid signal with a clear boundary and low signal on T1-WI and high signal on T2-WI. Diffusion-weighted imaging was used to diagnose acute-phase LI. WML, also known as "cerebral white matter hyperintensity," was first proposed by Hachinski, a Canadian neurologist, in 1987 [18]. The term refers to plaque-like or diffuse low-density or high-signal lesions in the bilateral white matter on computed tomography (CT) or T2-WI. WMLs appear as areas of low or equal signal on T1-WI and high signal on FLAIR and T2-WI. WML and LI diagnoses were evaluated by our hospital radiologist and confirmed by the same neurologist. Cerebral artery and carotid stenoses, as visualized by brain MRA or carotid color Doppler ultrasound, suggest artery stenosis.

Statistical analysis

General clinical data, Hcy, hs-CRP, carotid ultrasonography, and MRI results were recorded using Excel (Microsoft Corporation, Redmond, WA, USA). After logical checking and error correction, we used SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) to analyze the data. The measured data showed a normal distribution and were expressed as mean ± standard deviation. Independent-sample t-test was used for between-group comparisons for Hcy, hs-CRP, and serum creatinine levels. Variance analysis and multiple comparisons were used to compare Hcy levels in WML patients with different severities and to compare Hcy levels among LI patients and controls. Simple random sampling of physical examinees was performed using SAS version 9.3. Risk factors for ischemic CSVD were analyzed using a multivariate unconditional (bi-categorized) logistic regression analysis, and the regression model was assessed based on the receiver operating characteristic (ROC) curve. Identification of the segmentation points depended on the principle of maximized Youden index. P-values <.05 were considered statistically significant.

Results

The average Hcy level in 1,377 physical examinees was 12.39 ± 5.28 (range, 5–37.1) mmol/L. Table 2 shows the comparison of the clinical characteristics between patients with DCHS and patients with other lacunar syndromes (LIs). There was no significant difference between DCHS and other LIs except for dysarthria.

Table 3 shows the comparison of Hcy levels in WMLs with different severities, LI patients, physical examinees, and non-stroke patients. Patients with Fazekas 2–3 WML had significantly higher Hcy levels than those with Fazekas 0–1 WML (p=.0192). Patients with Fazekas 4–6 WML had significantly higher Hcy levels than those with Fazekas 2–3 WML (p=.0207). Patients in the LI group had significantly higher Hcy levels than non-LI patients (p=.001). Physical

Table 3. Comparison of Hcy levels in WML 0–1 patients, WML 2–3 patients, LI patients, physical examinees, and non-LI patients.

					95% CI	
Group	Serum Hcy level (mmol/L), mean ± SD	Mean difference	SE	Significance	Lower bound	Upper bound
WML 0 − 1 (n = 50)	11.39 ± 4.42	−3.687	1.0015	0.001[a]	−6.104	−1.27
WML 2 − 3 (n = 50)	12.33 ± 6.17	0.936	1.0459	0.019[b]	−1.588	3.46
WML 4 − 6 (n = 53)	15.08 ± 8.53	2.751	1.2179	0.075[c]	−0.188	5.69
LI (n = 103)	13.22 ± 4.93	2.715*	0.7649	0.001[d]	0.871	4.558
Physical examinees (n = 138)	12.36 ± 6.23	0.859	0.7832	0.821[e]	−1.029	2.747
Non-LI (n = 134)	10.51 ± 4.02	1.855	0.8371	0.080[f]	−0.157	3.868

[a]Patients with WML 0–1 vs patients with WML 4–6.
[b]patients with WML 0–1 vs patients with WML 2–3.
[c]patients with WML 2–3 vs patients with WML 4–6.
[d]LI patients vs non-LI patients.
[e]LI patients vs physical examinees.
[f]non-LI patients vs physical examinees.
Hcy, homocysteine; WML, white matter lesion; LI, lacunar infarction; SD, standard deviation; SE, standard error; CI, confidence interval.

Table 4. Comparison of hs-CRP levels in WML 0–1, WML 2–3, LI, and non-LI patients.

Group	hs-CRP level (mg/L), mean ± SD	Comparison	t-Value	*P*-value
WML 0 − 1[#] (n = 50)	2.79 ± 2.29	[#] and [†]	2.8	.0031*
WML 4 − 6[†] (n = 51)	4.76 ± 4.45			
LI group[&] (n = 103)	4.17 ± 3.6	[&] and [@]	2.4999	.0068*
Non-LI group[@] (n = 134)	2.78 ± 2.83			

*Statistically significant.
hs-CRP, high-sensitivity C-reactive protein; WML, white matter lesion; LI, lacunar infarction; SD, standard deviation.

Table 5. Multivariate unconditional logistic regression analysis of LI and non-LI.

Selected variables	β	SE	Wald χ^2	*P*-value	OR	95% CI
Age	0.063	0.014	19.342	.000	1.065	1.036, 1.095
Hypertension	0.410	0.129	10.173	.001	1.507	1.171, 1.939
Hyperhomocysteinemia	3.320	1.202	7.636	.006	27.668	2.625, 291.587
Increased IMT	1.424	0.451	9.963	.002	4.154	1.716, 10.058

Area under the receiver operating characteristics curve, 0.804 ($p<.001$; 95% CI, 0.736–0.872); maximum Youden index, 0.535; sensitivity and specificity on the best segmentation points, 80.8% and 72.7%, respectively; positive predictive valve, negative predictive value, and diagnostic accuracy rate of the regression model, 74.7%, 79.1%, and 76.7%, respectively.
LI, lacunar infarction; β, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval; IMT, intima media thickness.

examinees had slightly higher Hcy levels than non-LI patients (p=.080).

Table 4 shows the comparison of hs-CRP levels in Fazekas 0–1 WML, Fazekas 4–6 WML, LI, and non-LI patients. The hs-CRP levels in patients with Fazekas 4–6 WML were significantly higher than in patients with Fazekas 0–1 WML (p=.0031). LI patients had significantly higher hs-CRP levels than non-LI patients (p=.0068).

Three multivariate unconditional logistic regression analyses were performed. In the first analysis, sex (female = 0, male = 1), age, hypertension, diabetes, hyperlipidemia, hyperuricemia, increased IMT, cerebral artery and carotid stenoses, and anemia (no = 0, with = 1) were determined to be the independent variables, whereas LI was determined to be the dependent variable. The results showed that age, hypertension, hyperhomocysteinemia, and increased IMT were independently correlated with LI, whereas the remaining independent variables were not significantly correlated with LI (Table 5).

In the second analysis, the independent variables were the same as those in the first analysis, and severe WML was the dependent variable. The results showed that age, hypertension, increased IMT, and hyperhomocysteinemia were identified as risk factors for severe WML. The relationship between diabetes mellitus and severe WML was uncertain (the confidence interval included 1), and the remaining independent variables and severe WML were not significantly associated (Table 6). In the third analysis, sex (female = 0, male = 1), age, hypertension, diabetes, hyperlipidemia, increased IMT, cerebral artery and carotid stenoses, hyperhomocysteinemia, LI (no = 0, with = 1), and WML were determined to be the independent variables, and high hs-CRP level was determined to be the dependent variable. The results showed that LI, diabetes, and hyperhomocysteinemia independently correlated with high hs-CRP levels (Table 7).

Discussion

Our findings provide strong evidence that hyperhomocysteinemia is a risk factor for CSVD. A high Hcy level

Table 6. Multivariate unconditional logistic regression analysis of severe and non-severe WML cases.

Selected variables	β	SE	Wald χ^2	*P*-value	OR	95% CI
Age	0.064	0.015	18.062	.000	1.066	1.035, 1.097
Hypertension	0.336	0.145	5.367	.021	1.399	1.053, 1.858
Diabetes	0.644	0.352	3.344	.067	1.905	0.955, 3.799
Increased IMT	1.846	0.351	27.671	.000	6.333	3.184, 12.598
Hyperhomocysteinemia	0.685	0.311	4.847	.028	1.984	1.078, 3.651

Severe WML was defined as a total score $\geq$3.
Area under the receiver operating characteristics curve, 0.732 ($p<.001$; 95% CI, 0.658–0.807); maximum Youden index, 0.404; sensitivity and specificity on the best segmentation points, 67.2% and 73.2%, respectively; positive predictive valve, negative predictive value, and diagnostic accuracy rate of the regression model, 71.5%, 69.1%, and 70.2%, respectively.
β, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval; IMT, intima media thickness.

Table 7. Multivariate unconditional logistic regression analysis of cases with high hs-CRP and non-high hs-CRP.

Selected variables	β	SE	Wald χ^2	*P*-value	OR	95% CI
Hyperhomocysteinemia	1.375	0.573	5.764	.016	3.956	1.287, 12.155
LI	1.771	0.786	5.073	.024	5.876	1.259, 27.439
Diabetes	1.355	0.423	10.259	.001	3.878	1.692, 8.887

Area under the receiver operating characteristics curve, 0.762 ($p<.001$, 95% CI were 0.673-0.851); maximum Youden index, 0.377; sensitivity and specificity on the best segmentation points, 66.7% and 71.0%, respectively; positive predictive valve, negative predictive value, and diagnostic accuracy rate of the regression model, 69.7%, 68.1%, and 68.9%, respectively.
hs-CRP, high-sensitivity C-reactive protein β, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval; LI, lacunar infarction.

is a risk factor for stroke patients with cerebral white matter damage, and even mild hyperhomocysteinemia can significantly increase CSVD severity [19]. According to a report from India [20], 66% of all the patients with WMLs have comorbid hyperhomocysteinemia. However, reports on the relationship between Hcy level and WML severity are rare in China. Our results are similar to those reported outside China, which demonstrated that the Hcy level increases as WML severity increases [21]. Therefore, clinicians should pay more attention to hyperhomocysteinemia, as reducing it may decrease CSVD symptoms and delay its progress.

LI is a stroke caused by occlusion of a small artery. Acute LI in the corona radiata was significantly associated with DCHS [22]. DCHS was coined by Fisher [23] to identify a lacunar stroke characterized chiefly by the combination of dysarthria and "clumsiness" of one hand. DCHS is characterized by dysarthria, dysphagia, central facial weakness, deviation of the tongue on protrusion, incoordination of the affected hand, and mild imbalance on walking [24]. Our study showed that there was no significant difference between DCHS and other LIs except for dysarthria. DCHS is an infrequent (6% of lacunar strokes) lacunar syndrome [22], which generally occurs due to a focal lesion in the basal ganglia that damages corticofugal fibers as well as in the adjacent pontine neurons or their axons [12, 25]. Internal capsule and pons are the most frequent focal location of DCHS. CT/MRI may show evidence of a small, deep infarction in the territory of a penetrating vessel. As in other lacunar strokes, DCHS develops abruptly in the setting of a history of hypertension and shows good resolution [22, 26]. LIs that do not cause acute stroke symptoms are much more common than symptomatic ones [27].

LI increases the risk for cognitive decline and dementia [28]. Ischemic WML can cause cognitive impairment, and progression of ischemic WML is related to a parallel decline in cognitive function [29,30]. LI and ischemic WML have additive or synergistic effects on cognitive decline. Silent multiple LIs are associated with mild neuropsychological abnormalities, particularly in terms of the performance of executive functions and short delayed verbal memory [31]. Multiple silent LIs have a significant contribution to the risk of cognitive impairment and motor speed and executive function decline, whereas global cognitive functions and memory functions remain unaffected [32]. In the early stages of CSVD, mild neuropsychological abnormalities appear to be related to LI rather than to WML [31]. LI lesion load is the most important MRI parameter associated with cognitive dysfunction [33]. Ischemic WML can cause varying degrees of cognitive impairment, which is mainly manifested as decline in visuospatial and executive functions, attention, and information processing speed and delayed recall, whereas paraventricular and deep WMLs may manifest as dysfunctions of different cognitive domains [34]. As the scope and severity of WML and LI increase, the affected nerve conduction loop expands, which leads to more cognitive domain impairments. Our comparison of the Hcy levels between patients with and without LI clearly reflected the effects of Hcy on small cerebral arteries. Currently, only a few studies have compared Hcy levels between

LI patients and hospitalized patients without LI. Similar to the findings of prior studies, our results showed that Hcy levels were significantly higher in the LI group than in physical examinees. Our research further demonstrated that a high Hcy level is a risk factor for LI.

Only a few reports have investigated the relationship between hs-CRP and WMLs of different severities. Our findings confirm that the severity of WMLs is related to hs-CRP levels. hs-CRP, which is elevated in more severe ischemic WMLs [35,36], can activate the monocyte–macrophage arch and the complement system, leading to vascular endothelial cell damage and release of inflammatory cytokines that promote atherosclerosis [36] and cause leukoencephalopathy. Thus, elevated hs-CRP levels are a risk factor for recurrent stroke and other vascular events [37].

We observed elevated hs-CRP in patients with LI and severe WML, and analysis revealed that hyperhomocysteinemia was associated with high hs-CRP levels. Inflammation is an important pathophysiological mechanism of LI and WML. Hcy induces an increase in the levels of hs-CRP through the NMDAr-ROS-ERK1/2/p38-NF-κB signaling pathway and initiates an inflammatory reaction in vascular smooth muscle cells [38]. Elevated hs-CRP induces vascular smooth muscle cells to produce interleukin 6 and inhibits the expression of peroxisome proliferator–activated receptor gamma to induce inflammation *via* the myeloid differentiation factor 88–independent TLR4 signaling pathway (TLR4/IRF3/NF-κB) [10]. Elevated hs-CRP could promote adhesion of monocytes to endothelial cells *via* NADPH oxidase–mediated oxidative stress, and enhanced monocyte adhesion to endothelial cells is an early event in atherogenesis [39]. The inflammatory mechanism is very complicated, and further studies are needed to confirm and understand the mechanism.

A possible limitation of this study is the setting, i.e. a single-center study from southern China. Therefore, the generalizability of the results may be limited. Multicenter studies must be conducted in the future to corroborate the results. An indispensable line of research in the future would be to assess whether hyperhomocysteinemia is a predictor of vascular cognitive impairment in CSVD.

In conclusion, the assessment of Hcy levels is important for the prevention and treatment of ischemic CSVD. Hyperhomocysteinemia is a risk factor for LI and severe WML, and higher Hcy levels correlated with more severe WMLs. Hyperhomocysteinemia is significantly correlated with high hs-CRP levels; however, further research is needed to confirm and understand the inflammatory mechanism underlying the association between hyperhomocysteinemia and ischemic CSVD.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Statement of ethics

The study design was approved by the ethics review board of the 3rd Affiliated Hospital of Shenzhen University (approval no. 2018LHYYSJNL-003-01). The subjects (or their parents or guardians) provided written informed consent.

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