Study of the Inflammatory Mechanisms in Hyperhomocysteinemia on Large-Artery Atherosclerosis Based on Hypersensitive C-Reactive Protein—A Study from Southern China

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Objective: To study the inflammatory mechanism of hyperhomocysteinemia on large-artery atherosclerosis based on hypersensitive C-reactive protein in patients. *Methods:* In all, 153 inpatients and 1357 physical examinees were selected. The levels of homocysteine were compared between the carotid/intracranial artery stenosis group and the nonstenosis group, between the carotid artery unstable plaque group and the nonplaque group, and between the intima-media thickness (IMT) greater than or equal to 1 group and the normal IMT group. The hypersensitive C-reactive protein levels were compared between the lacunar infarction (LI) group and the nonstroke control group and between the unstable plaque group and the nonplaque group. *Results:* Homocysteine level was significantly higher in the carotid/intracranial artery stenosis group than in the nonstenosis group, in the LI group than in the inpatient nonstroke group, and in the IMT greater than or equal to 1 group than in the normal IMT group. The hypersensitive C-reactive protein level was significantly higher in the LI group than in the nonstroke group and in the unstable plaque group than in the nonplaque group. *Conclusions:* Hyperhomocysteinemia may aggravate the development of IMT, carotid atherosclerotic plaque instability, and carotid/intracranial artery stenosis by increasing inflammation, ultimately leading to the occurrence of LI. Hyperhomocysteinemia-induced inflammation mechanism warrants further study.

Key Words: Hyperhomocysteinemia—large-artery atherosclerosis—hypersensitive C-reactive protein—intracranial artery stenosis—carotid artery unstable plaque—carotid intima-media thickness

Introduction

The mortality rate and disability rate of stroke are very high, which has a great influence on the quality of life of patients. It is the third most common reason that influences the disability adjusted life years[1] and bring serious burdens to families and the society. The report of China Cardiovascular Disease (2017)[2] indicates that there are 13 million cases of stroke in China, and studies on the global disease burden show that stroke has become the first cause of death in China since 2010. Ischemic stroke accounts for 67.3%-80.5% of stroke patients,[3] and lacunar infarction (LI) is the most common subtype of ischemic stroke (54.1%).[4]

Hyperhomocysteinemia has been identified as an important risk factor for atherosclerosis, but its influence on intracranial and extracranial arterial stenosis is still

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The study design was approved by the appropriate ethics review board.

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controversial. Foreign studies have reported[5,6] that hyperhomocysteinemia only plays an important role in cerebral ischemia of small blood vessels, rather than in atherosclerosis of large arteries. We found that hyperhomocysteinemia is closely related to aortic arteriosclerosis in clinical work.[7] Homocysteine increases the risk of ischemic stroke, and inflammation plays an important role in the process of ischemic stroke.[8] The levels of inflammatory cytokines in[9] individuals with hyperhomocysteinemia are elevated. Hypersensitive C-reactive protein (hs-CRP) is a commonly used clinical inflammatory marker, which plays an important role in the inflammatory response to atherosclerosis.[10] Research shows that inflammation occurs before the onset of stroke and may also be the cause of stroke.[11]

We studied the relationship between carotid intima-media thickness (IMT), carotid ultrasound plaque, intracranial/extracranial artery stenosis, and homocysteine and hs-CRP in patients with hospitalized (or without hospitalized) in our department of neurology. We analyzed the effect of hyperhomocysteinemia on large-artery atherosclerosis to study the changes in hs-CRP in hyperhomocysteinemia causing large-artery atherosclerosis.

Materials and Methods

Study Participants

A total of 153 patients admitted to the Department of Neurology of our hospital and 1357 physical examinees from January, 2017 to February, 2018 were selected in our hospital. Among these 153 patients, 80 of whom are males and 73 are females, all aged between 26 and 90 years. There were 67 inpatients having LI, 17 with other types of cerebral infarction, and 69 nonstroke patients. Infarction was defined according to Trial of Org 10 172 in Acute Stroke Treatment (TOAST) classification[12] standard.

The hospitalized patients with various common diseases included: 90 inpatients with hypertension, 43 with diabetes mellitus, 3 with impaired glucose regulation, 62 with hyperlipidemia, 31 with hyperuricemia, 4 with atrial fibrillation, 14 with coronary heart disease, and 6 with other arrhythmia disorders.

The number of patients who were mainly diagnosed with nonstroke disease included: 45 inpatients with posterior circulation ischemia, 8 with peripheral neuropathy, 5 with benign paroxysmal positional vertigo, 3 with transient ischemic attack, 2 with tension headache, 1 with spinocerebellar ataxia, 1 with syncope, 1 with Moyamoya disease, 1 with epilepsy, and 1 with Meniere disease. The detailed records of the patients' underlying diseases were collected. All hospitalized patients underwent magnetic resonance imaging (MRI), including T2-weighted imaging (T2WI), fluid attenuated inversion recovery, magnetic resonance angiography, and other relevant tests. All patients were subjected to plasma homocysteine (homocysteine), high-sensitivity C-reactive protein (hs-CRP), carotid artery color Doppler ultrasonography, and other examinations.

Inclusion and Exclusion Criteria for Patients

Patients were included based on the following criteria:

(1) those between 30 and 90 years old;
(2) who did not have infectious diseases, such as pulmonary infection and urinary system infection;
(3) who had not suffered from severe craniocerebral trauma and sequelae; malignant tumors; heart, liver, kidney, lung or other organ failure; or immune diseases in active stage;
(4) who had no terminal-stage illness;
(5) who did not have white matter lesions (WMLs) caused by central nervous system poisoning, hereditary degeneration, metabolic diseases, and hydrocephalus.

Technical Methods

The diagnostic techniques used in this study include:

(1) Image acquisition and evaluation of WML: Siemens 1.5-T magnetic resonance was used for routine brain MRI scans, including T1-weighted imaging, T2WI, and T2-fluid attenuated inversion recovery sequences with layer thickness of 5 mm and layer spacing of 1.5 mm.
(2) Detection of homocysteine and hs-CRP: All patients were fed a nonhigh protein diet one day before blood collection. The blood samples of elbow veins were collected for homocysteine and hs-CRP over 12 hours after fasting. Homocysteine was detected by the Beckman Au5821 automatic biochemical analyzer and was measured by the rate method. The kit was provided by Jinhua Qiangsheng Biotechnology Co., Ltd. The reference range of homocysteine is 5-15 mcmol/L, and >15 mcml/L is considered to be hyperhomocysteinemia.
(3) Color Doppler ultrasound examination of cervical vessels: Philips iE Elite ultrasonic diagnostic instrument was used with the probe frequency of 10-15 MHz. The IMT of the carotid artery was recorded. Carotid IMT is defined as the vertical distance between the upper edge of the intima and the lower edge of the medial membrane. The carotid atherosclerotic plaque and the location, size, shape, and echo characteristics of the plaque were examined and recorded.

Diagnostic Criteria

Carotid Ultrasound Criteria for IMT and Unstable Plaques

(a) Criteria for IMT thickening and the definition of plaque by carotid ultrasound: Ultrasound diagnostic criteria for carotid endometrial lesions[13] include two-dimensional gray-scale ultrasonography, which showed intima-media fusion with uneven echo changes. When IMT was greater than or equal to 1 mm, it was considered to be thickened. Plaque is defined as a local eminence protruding (from the arterial lumen) greater than 0.5 mm or more than 50% of the surrounding IMT values or IMT greater than 1.5 mm.[14]

(b) Judgment of the nature of plaque and stability by carotid ultrasound: According to the difference between plaque echo and vessel wall echo intensity, a plaque can be divided into: (1) hypoechoic plaque: the echo of the plaque is lower than that of the intima layer; (2) moderate echo plaque: the echo of the plaque is relatively consistent with that of the intima layer; and (3) hyperechoic plaque: the echo of plaque is equal to or slightly higher than that of the outer membrane layers. The medium or moderate low echo is soft plaque, while the strong echo plaque or calcified plaque with sound shadow is hard plaque. The mixed plaque has uneven echo intensity, with both soft plaque and hard plaque. Soft plaque and mixed plaque are unstable plaques. Hard plaques are also called calcified plaques as stable plaques.[15] Calcification is common in stable plaques and helps to determine their stability. Ultrasound is highly sensitive and specific in detecting plaque calcification and degree. All carotid artery ultrasound examinations were performed independently by an experienced ultrasound physician, and the results were aggregated and confirmed by another advanced ultrasound physician.

Diagnostic Criteria for Various Diseases

Hypertension was diagnosed with reference to the criteria of the 2010 edition of China Hypertension Guidelines.[16] Type 2 diabetes was diagnosed with reference to the Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes (2017 edition).[17] Hyperlipidemia was diagnosed with reference to the diagnostic criteria[18] in the Chinese Adult Guidelines for the Prevention and Treatment of Abnormal Blood Lipids (2007), while hyperuricemia was diagnosed hyperuricemia based on the Chinese Expert Consensus on the diagnosis and treatment of asymptomatic hyperuricemia with cardiovascular diseases.[19] The diagnosis of cerebral infarction was done with reference to the diagnostic criteria of Guidelines for the Prevention and Treatment of Cerebrovascular Diseases in China.[20]

LI is defined as a small infarct located in the deep part of the brain with a size of 2-20 mm. It occurs mostly in the basal ganglia, thalamus, and brainstem. LI shows cerebrospinal fluid signal on MRI, with clear boundary, that is, low signal on T1-weighted imaging and high signal on T2WI. For acute early LI, it needs to be judged by diffusion weighted imaging. The diagnosis of LI was evaluated by a fixed radiologist and confirmed by a specialist neurologist.

Ethical Approval Statement

The study design was approved by the appropriate ethics review board (NO. 2018LHYYSJNL-003-01).

Statistical Analysis

The general clinical data, blood biochemical results, carotid ultrasonography, and MRI results were recorded on Excel software. After logical checking and error correction, the data were analyzed by SAS9.3 statistical software. The measured data conformed to normal distribution and were expressed by mean ± standard deviation. The t test of independent samples was used for comparison between groups, and the difference ($P < .05$) was statistically significant.

Results

During the period from January 2017 to February 2018, the homocysteine levels of 1357 (aged 31-90 years) patients who underwent physical examination in our hospital were as follows: homocysteine (12.39 ± 5.28) mcmol/L, homocysteine range (5-37.1) mcmol/L.

Homocysteine Levels in LI

Homocysteine was compared between the LI group, the nonstroke inpatients, and the physical examinees (Tables 1 and 2). Homocysteine in the LI group was significantly higher than that in the physical examination group ($P < .05$) and in the nonstroke group ($P < .05$) (Table 3).

Influence of Homocysteine on Carotid Atherosclerosis (IMT, Carotid Plaque, Vascular Stenosis)

The homocysteine levels in the IMT hypertrophy group were significantly higher than that in the normal IMT group ($P < .05$) (Table 4). Homocysteine in the unstable plaque group was significantly higher than that in the nonplaque group ($P < .05$).

It was revealed that homocysteine in the patients with intracranial/external artery stenosis group was significantly higher than that in the patients without artery stenosis group ($P < .05$).

Status of hs-CRP Status of Unstable Plaques and LI Patients (Table 5)

We found that hs-CRP in the unstable plaque group was significantly higher ($P < .05$) than that in the

Table 1. *Distribution of the number of men and women (physical examination group) in different age groups (unit: person)*

Grouping by age (years)	31-40	41-50	51-60	61-70	71-80	81-90
Men	34	161	305	160	41	10
Woman	42	118	286	175	37	8
Total	76	279	591	335	78	18

Table 2. *Distribution of male and female inpatients in different age groups (unit: person)*

Grouping by age (years)	26-30	31-40	41-50	51-60	61-70	71-80	81-90
Men	2	3	7	20	20	14	12
Woman	1	3	10	14	26	13	7
Total	3	6	17	34	46	27	19

nonplaque group (Table 5). This study also showed that hs-CRP in the LI group was significantly higher than that in the hospitalized nonstroke group ($P < .05$).

Discussion

Effect of Hyperhomocysteinemia on Carotid Atherosclerosis (IMT, Unstable Plaque and Stenosis)

Carotid ultrasound is an effective, noninvasive, and reproducible tool for the diagnosis of atherosclerosis.[21] Carotid atherosclerosis can reflect cerebral atherosclerosis to some extent and is an important predictor of cerebral infarction.[22] The previous study[23] confirmed that the incidence of severe and unstable carotid atherosclerosis was higher in patients with ischemic stroke. Hyperhomocysteinemia is an independent risk factor for carotid atherosclerosis and is significantly related to the degree of carotid atherosclerosis. Our results suggest that hyperhomocysteinemia may be involved in IMT hypertrophy. IMT hypertrophy is the pathological manifestation of atherosclerosis. It is an effective marker for predicting preclinical atherosclerosis and cardiovascular events.

Table 3. *Comparison of homocysteine between the LI group, physical examination group, and the nonstroke inpatient group (mean ± SD)*

Group	n	Serum homocysteine level (mcmol/L)	t	*P*
1. LI group	66	13.17 ± 5.8	1&2: 1.7336	.0438
2. Physical examination group	1357	11.92 ± 3.72		
3. Nonstroke inpatient	69	10.88 ± 3.98	1&3: 2.6634	.0043

LI, lacunar infarction.

Comparison of homocysteine between the LI group and the physical examination group showed that $P < .05$. Comparison of homocysteine between the LI group and the hospitalized nonstroke patients showed that $P < .05$.

Table 4. *Comparison of homocysteine between patients with IMT ≥1/IMT<1 and with/without unstable carotid plaque groups (mean ± SD)*

Group	n	Serum homocysteine level (mcmol/L)	t	*P*
1. IMT≥1group	57	15.93 ± 11.02	1&2: 2.8492	.0029
2. IMT normal group	94	11.53 ± 4.89		
3. Unstable plaque group	34	12.59 ± 4.48	3&4: 2.6798	.0046
4. No plaque group	37	10.03 ± 3.6		
5. Arterial stenosis group	21	14.05 ± 4.89	5&6: 2.6714	.0054
6. No arterial stenosis group	23	10.74 ± 3.02		

IMT, arterial medial thickness.

Comparison of homocysteine between the IMT ≥ 1 group and the normal IMT group, $P < .05$.

Comparison of homocysteine in the unstable plaque group (19 people with soft plaques and 15 people with mixed plaques) was compared to that in the nonplaque group, $P < .05$

Comparison of homocysteine between the stenosis group and the nonstenosis group showed that $P < .05$. Arterial stenosis is defined as MRA discovery of intracranial artery stenosis or carotid artery color Doppler ultrasound discovery of carotid artery stenosis.

Table 5. *Comparison of hs-CRP between the unstable plaque groups and plaque-free group and between LI group and nonstroke inpatients (mean ± SD)*

Group	n	hs-CRP(mg/L)	t	*P*
1. Unstable plaque group	34	4.24 ± 3.73	1&2: 2.3893	.0098
2. Plaque free group	37	2.49 ± 2.17		
3. LI group	66	4.17 ± 3.6	3&4: 2.4999	.0068
4. Nonstroke control group	69	2.78 ± 2.83		

LI, lacunar infarction.

hs-CRP of the unstable plaques group (19 with soft plaques and 15 with mixed plaques) was compared with that of the nonplaques group, $P < .05$.

Comparison of hs-CRP between the LI group and the nonstroke inpatient group showed that $P < .05$.

A long-term follow-up study[24] found that preclinical atherosclerosis (increased IMT or asymptomatic carotid plaque) increased the risk of cardiovascular events. Previous studies[25] have shown that carotid atherosclerosis increases the risk of stroke recurrence.

Effect of Hyperhomocysteinemia on Unstable Carotid Plaque and Its Inflammatory Mechanism

Unstable carotid plaque is characterized by a large number of inflammatory reactions, including large lipid nuclei, thin fibrous caps, intimal denudation and surface platelet aggregation, plaque rupture, and neovascularization. The echo characteristics of carotid plaques were significantly correlated with the recurrence of stroke[26] (P= .0028). Fisher[27] believed that unstable plaque rupture was the most important cause of cerebral infarction; the more unstable plaques, the higher the probability of LI. Our study indicated that hyperhomocysteinemia might cause unstable plaques in the carotid artery. The increased plasma homocysteine level in patients with carotid artery disease was significantly correlated with the increased incidence of acute cerebral infarction.[28] The significantly higher levels of hs-CRP in the unstable plaque group compared to the nonplaque group indicated that patients in the unstable plaque group had more severe inflammatory response. Thus, hyperhomocysteinemia may lead to the occurrence and development of unstable carotid artery plaques by increasing inflammatory response. Previous studies[9] have confirmed that the level of inflammatory cytokines in individuals with hyperhomocysteinemia increases, and hyperhomocysteinemia causes vascular remodeling through inflammatory response. The mechanisms of hyperhomocysteinemia-induced vascular injury[29] include endothelial cell damage, DNA dysfunction, smooth muscle cell proliferation, increased oxidative stress, reduced glutathione peroxidase activity, and increased inflammation. This study showed that hyperhomocysteinemia was involved in the inflammatory process (hs-CRP elevation), which resulted in the increase of unstable carotid plaques and the occurrence and development of LI. Therefore, homocysteine increases the risk of ischemic stroke, and inflammatory response plays an important role in the process of ischemic stroke.[8]

Effect and Mechanism of Hyperhomocysteinemia on Intracranial and Extracranial Artery Stenosis

Previous studies[6] have shown that that there is no significant correlation between homocysteine level and large vessel disease (P= .075), and hyperhomocysteinemia is an independent risk factor for cerebral small vessel disease ($P < .001$). Chinese studies[30] believe that hyperhomocysteinemia is related to the severity of carotid artery stenosis. The Chinese research reports[31] that moderate to severe carotid stenosis in patients with cerebral infarction were significantly higher in patients with hyperhomocysteinemia than in patients with mild stenosis of carotid artery ($P < .05$). Homocysteine has a close relationship with the increased intracranial/external artery stenosis as our study also showed that the patients with intracranial/external artery stenosis group have higher levels of homocysteine than that the patients without artery stenosis group. It has been reported that hyperhomocysteinemia has different effects on cerebral large-artery atherosclerosis in China[30] and Republic of Korea.[5] Whether this difference is related to race and region is still unclear. The exact mechanism of hyperhomocysteinemia leading to intracranial/external arterial stenosis is still unclear. Studies suggest that the mechanisms may be as follows: (1) hyperhomocysteinemia accelerates endovascular cell proliferation and collagen deposition, aggravates vascular contraction and remodeling, thus leading to arteriosclerosis.[32] Angiotensin II also induces collagen synthesis in adventitia fibroblasts[33]; (2) Hyperhomocysteinemia induces superoxide increase, secondary vascular dysfunction, and vascular smooth muscle and elastin content, causing atherosclerosis[34]; (3) Hyperhomocysteinemia can lead to atherosclerosis through inflammation.[13] Thus, hyperhomocysteinemia can promote cerebral small vessel disease (LI, WML).[5]

Effect of Hyperhomocysteinemia on LI

According to Trial of Org 10 172 in Acute Stroke Treatment classification,[12] cerebral infarction can be divided

into 5 types, and the etiology of each type of stroke is significantly different. LI belongs to small artery occlusion type. The comparison of homocysteine of LI with those who underwent physical examination can better reflect the effect of homocysteine on cerebral arterioles. Currently, there are few studies comparing homocysteine of small artery occlusion (LI) patients with physical examinees or hospitalized patients without stroke. The homocysteine levels of the LI group in our study were significantly higher than those in the physical examination and the nonstroke groups; thus, this study supports the view that homocysteine was a risk factor for LI. Serum Hcy level increases in patients with acute cerebral infarction,[35] and the level of Hcy is directly related to inflammatory factors, nerve factors, and NO metabolism.

Levels of hs-CRP in Unstable Carotid Plaque and LI

The levels of hs-CRP can reflect the composition of atherosclerotic plaques and predict the possibility of plaque rupture.[36] The studies[37] show that hs-CRP in serum are significantly associated with homocysteine concentration. A previous study[38] showed that hs-CRP was closely related to carotid plaque and IMT. Hs-CRP could be used as a highly sensitive index to judge the risk and prognosis of atherosclerotic cerebral infarction. Since hs-CRP was found to be higher in the unstable plaque group compared to the nonplaque group, we believe that hs-CRP was closely related to carotid unstable plaques. An earlier study[10] have confirmed that CRP stimulates vascular smooth muscle cells (VSMCs) to produce IL-6 and inhibits the expression of peroxisome proliferator-activated receptor gamma through myeloid differentiation factor 88 independent TLR4 signaling pathway (TLR4/IRF3/NF-kappa B) to induce inflammation. Another study[39] confirmed that homocysteine mediates CRP production through NMDAr-ROS-ERK1/2/p38-NF-kappa B signaling pathway, thus triggering inflammatory response in VSMCs.

The Mechanism Between Hyperhomocysteinemia With Inflammatory Reaction

Serum homocysteine level correlates to inflammatory/immune factors. Inflammation is a complex vascular response that has evolved to eliminate infection and to repair injured tissue. Failure of an inflammatory response to resolve has become recognized as a major contributor to the pathology of diverse diseases (including acute brain injuries). A previous study[40] shows that proinflammatory factors (IL-1 beta, IL-6, tumor necrosis factor (TNF)-alpha) play an important role in the hyperhomocysteinemia model, which may be related to the pathogenesis of ischemic cerebrovascular disease (IL, cWML). Hyperhomocysteinemia can increase the inflammatory response of the body through the increase of proinflammatory factors IL-1 beta, IL-6, and TNF-alpha and cause atherosclerosis and stroke.[41] IL-1 is a proinflammatory cytokine and key contributor that causes damage after acute brain injury.[42] IL-1 expression in the brain increases in response to acute and chronic insults, and IL-1 contributes directly to experimentally induced ischemic, excitotoxic, and traumatic brain injury. Regulation of IL-1 beta production is essential for the development of new drugs to treat brain injury. The IL-1 receptor antagonist (IL-1ra) has shown to modulate the proinflammatory cytokine cascade by blocking the binding of IL-1 to its signaling receptor. Blockage of IL-1 signaling by elevated levels of IL-1ra has a neuroprotective effect and improves neurological recovery after traumatic brain injury.[43] The naturally occurring IL-1ra is currently being considered for the treatment of stroke and other disorders.[44] Probucol prevents L-homocysteine-induced inflammatory monocytes differentiation and reactive oxygen species generation probably through inhibiting NADPH oxidase activity.[45] A previous study[46] demonstrate that Hcy may trigger inflammation through inhibiting cystathionine γ-lyase (CSE)-H2S signaling, associated with increased promoter DNA methylation and transcriptional repression of CSE in macrophages. Thus, Hcy regulates endothelin type A (ETA) receptor expression via the Sirt1/ERK1/2 signaling pathway in VSMCs. Hcy upregulated blood pressure through Sirt1/ERK1/2/ETA receptor pathway.[47] Based on the above mentioned results from different studies and the results of our study, we speculate that Hyperhomocysteinemia leads to large-artery atherosclerosis through inflammatory pathogenesis, secondary stroke; However, the specific inflammatory mechanism is yet unclear. Inflammation can promote atherosclerotic plaque formation, increase plaque instability, plaque progression and rupture[48] and form cerebral vascular occlusion. As the hs-CRP in the LI group was significantly higher than that in the hospitalized nonstroke group, our research results support the theory that hyperhomocysteinemia may induces/promotes LI through inflammation.

A possible limitation of the research may be that it is a single-center study and therefore, the generalizability of the results may be limited. Additionally, a multicenter study must be conducted in future research to validate our findings.

Conduct research on hyperhomocysteinemia animal models for further elucidation of the inflammatory mechanisms in hyperhomocysteinemia on large-artery atherosclerosis.

In conclusion, our study suggests that hyperhomocysteinemia may aggravate the development of IMT, unstable carotid atherosclerotic plaque, and carotid/intracranial artery stenosis by increasing the inflammatory response and may ultimately lead to the occurrence of LI. Paying attention to the prevention and treatment of hyperhomocysteinemia may help reduce the occurrence of large-artery atherosclerosis and LI formation. Inflammatory pathogenesis of large-artery atherosclerosis caused by hyperhomocysteinemia needs more further hyperhomocysteinemia animal model study.

Conflict of Interest

There are no conflicts of interest to declare.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.04.021.

References

1. Murray CJ, Lopez AD. Measuring the global burden of disease. N Engl J Med 2013;369:448-457 https://doi.org/10.1056/NEJMra1201534.
2. Chen WW, Cao RL, Liu LS, et al. Summary of Chinese cardiovascular disease report 2017. Chin J Circ 2008;33:1-8.
3. Feigin VL, Lawes CM, Bennett DA, et al. Stroke epidemiology: a review of population -based studies of incidence, prevalence, and case-fatality in the late 20th century. Lancet Neurol 2003;2:43-53 https://doi.org/10.1016/S1474-4422(03)00266-7.
4. Turin TC, Kita Y, Rumana N, et al. Ischemic Stroke subtypes in a Japanese population: Takashima Stroke Registry, 1988−2004. Stroke 2010;41:1871-1876 http://dx.doi.org/10.1161/STROKEAHA.110.581033.
5. Park SY, An SA, Lee HB, et al. Different impact of hyperhomocysteinemia on cerebral small vessel ischemia andcervico-cerebral atherosclerosis in non-stroke individuals. Thromb Res 2013;131:e12-e16 https://doi.org/10.1016/j.thromres.2012.11.011.
6. Feng C, Bai X, Xu Y, et al. Hyperhomocysteinemia associates with small vessel disease more closely than large vessel disease. Int J Med Sci 2013;10:408-412 https://doi.org/10.7150/ijms.5272.
7. Cao LM, Guo Y, Guan JW, et al. Distribution characteristics and early warning value of homocysteine in different populations. Chin J Pract Nerv Dis 2015;18:35-36.
8. Banecka-Majkutewicz Z, Sawuła W, Kadziński L, et al. Homocysteine, heat shock proteins, genistein and vitamins in ischemic stroke-pathogenic and therapeutic implications. Acta Biochim Pol. 2012;59:495-499.
9. Nenseter MS, Uhland T, Retterstøl K, et al. Dysregulated RANK ligand/RANK axis in hyperhomocysteinemic subjects: effect of treatment with B-vitamins. Stroke 2009;40:241-247 https://doi.org/10.1161/STROKEAHA.108.522995.
10. Liu N, Liu JT, Ji YY, et al. C-reactive protein triggers inflammatory responses partly via TLR4/IRF3/NF-κB signaling pathway in rat vascular smooth muscle cells. Life Sci 2010;87:367-374 https://doi.org/10.1016/j.lfs.2010.07.012.
11. Chiba T, Itoh T, Tabuchi M, et al. Interleukin-1b accelerates the onset of stroke in stroke-prone spontaneously hypertensive rats. Mediators Inflamm 2012;2012:701976 https://doi.org/10.1155/2012/701976.
12. Adams Jr HP, Bendixen BH, Kappelle LJ. Classification of subtype of acute ischemic stroke: definitions for use in a multicenter clinical trial. Stroke 1993;24:35-41.
13. Wu JT, Wu LL. Linking inflammation and atherogenesis: soluble markers identified for the detection of risk factors and for early risk assessment. Clin Chim Acta 2006;366:74-80 https://doi.org/10.1016/j.cca.2005.10.016.
14. Touboul PJ, Henneriei MG, Meairs S, et al. Mannheim carotid intimamedia thickness and consensus (2004-2006). Cerebrovasc Dis 2007;23:75-80.
15. Yamagishi M, Terashima M, Awano K, et al. Morphology of vulnerable coronary plaque: insights from follow-up of patients examined by intravascular ultrasound before an acute coronary syndrome. J Am Coll Cardiol 2000;35:106-111 https://doi.org/10.1016/S0735-1097(99)00533-1.
16. Wang W. New points of view of the 2010 edition of China hypertension guidelines. Chin Community Doct 2011;24:10.
17. Diabetes Society of Chinese Medical Association. Guidelines for the prevention and treatment of type 2 diabetes in China (2017 edition). Chin J Diabetes Mellit 2018;10:4-67.
18. Xu H, Wu ZM, Lu ZL. Summary and interpretation of the guidelines for prevention and treatment of adult blood lipid abnormalities in China (2007). Chin J Geriatr Cardiovasc Cerebrovas Dis 2008;10:238-240.
19. Chinese Medical Association Cardiovascular Internal Medicine Branch. Chinese Medical Association Evidence-based Medicine Professional Committee. Expert consensus on the diagnosis and treatment of asymptomatic hyperuricemia with cardiovascular diseases. Chin Gen Pract 2010;26:1145-1149.
20. Department of Disease Prevention and Control. Ministry of Health. Society of Neurology. Chinese Medical Association. Guidelines for the prevention and treatment of cerebrovascular diseases in China. Beijing: People's Health Publishing House; 2007; 47-48.
21. Katakami N, Kaneto H, Shimomura I. Carotid ultrasonography: a potent tool for better clinical practice in diagnosis of atherosclerosis in diabetic patients. J Diabetes Investig 2014;5:3-13 https://doi.org/10.1111/jdi.12106.
22. Li GW, Zheng GY, Li JG, et al. Relationship between carotid atherosclerosis and cerebral infarction. Chin Med Sci J 2010;25:32-37 https://doi.org/10.1016/S1001-9294(10)60017-X.
23. Zhang AJ, Zhang AY, Zhong C. Carotid atherosclerosis in ischemic cerebrovascular patients. J Clin Med Res 2009;1:40-44 https://doi.org/10.4021/jocmr2009.03.1226.
24. Novo S, Peritore A, Trovato RL, et al. Preclinical atherosclerosis and metabolic syndrome increase cardio-and cerebrovascular events rate: a 20-year follow up. Cardiovasc Diabetol 2013;12:155 https://doi.org/10.1186/1475-2840-12-155.
25. Liu J, Zhu Y, Wu Y, et al. Association of carotid atherosclerosis and recurrent cerebral infarction in the Chinese population: a meta-analysis. Neuropsychiatr Dis Treat 2017;13:527-533 https://doi.org/10.2147/NDT.S124386.
26. Singh AS, Atam V, Jain N, et al. Association of carotid plaque echogenicity with recurrence of ischemic stroke. N Am J Med Sci 2013;5:371-376 https://doi.org/10.4103/1947-2714.114170.
27. Fisher M, Paganini-Hill A, Martin A, et al. Carotid plaque pathology:thrombosis, ulceration,and stroke pathogenesis. Stroke 2005;36:253-257 http://dx.doi.org/10.1161/01.STR.0000152336.71224.21.
28. Wu B, Lin S, Hao Z, et al. Proportion, risk factors and outcome of lacunar infarction: a hospital-based study in a Chinese population. Cerebrovasc Dis 2010;29:181-187 https://doi.org/10.1159/000267277.
29. Pushpakumar S, Kundu S, Sen U. Endothelial dysfunction: the link between homocysteine and hydrogen sulfide. Curr Med Chem 2014;21:3662-3672 https://doi.org/10.2174/0929867321666140706142335.

30. Liu Y, Zhang P, Bi Q. The relationship between hyperhomocysteinemia and carotid artery stenosis in elderly stroke patients. Chin J Geriatr Heart Brain Ves Dis 2008;10:527-528.
31. Xu H. The relationship between plasma homocysteine level and carotid atherosclerosis in patients with cerebral infarction. Shandong Med J 2010;50:43-44.
32. Guo YH, Chen FY, Wang GS, et al. Diet-induced hyper -homocysteinemia exacerbates vascular reverse remodeling of balloon-injured arteries in rat. Chin Med J (Engl) 2008;121:2265-2271 http://dx.doi.org/10.1097/00029330-200811020-00011.
33. Yao D, Sun NL. Hyperhomocysteinemia accelerates collagen accumulation in the adventitia of balloon-injured rat carotid arteries via angiotensin II type 1 receptor. Int J Mol Sci 2014;15:19487-19498 https://doi.org/10.3390/ijms151119487.
34. Dayal S, Baumbach GL, Arning E, et al. Deficiency of superoxide dismutase promotes cerebral vascular hypertrophy and vascular dysfunction in hyperhomocy-steinemia. PLoS One 2017;12:e0175732 https://doi.org/10.1371/journal.pone.0175732.
35. Zhi GL, Xia Z, Gui BW. Serum homocysteine level in patients with acute cerebral infarction and its correlation with inflammatory factors, nerve factors and NO metabolism. J Hainan Med Univ 2017;23:147-150.
36. Haim M, Benderly M, Tanne D, et al. C-reactive protein, bezafibrate, and recurrent coronary events in patientswith chronic coronary heart disease. Am Heart J 2007;154:1095-1101 https://doi.org/10.1016/j.ahj.2007.07.026.
37. Li TY, Chen Y, Li J, et al. Serum homocysteine concentration is significantly associated with inflammatory/immune factors. PLoS ONE 2015;10:e0138099. https://doi.org/10.1371/journal.pone.0138099.
38. Zhao L, Zhai Z, Hou W. Analysis of carotid color ultrasonography and high sensitive C-reactive protein in patients with atherosclerotic cerebral infarction. Pak J Med Sci 2016;32:931-934.
39. Pang X, Liu J, Zhao J, et al. Homocysteine induces the expression of C-reactive protein via NMDAr-ROS-MAPK-NF-κB signal pathway in rat vascular smooth muscle cells. Atherosclerosis 2014;236:73-81 https://doi.org/10.1016/j.atherosclerosis.2014.06.021.
40. Chiba T, Itoh T, Tabuchi M, et al. Interleukin-1b accelerates the onset of stroke in stroke-prone spontaneously hypertensive rats. Mediators Inflamm 2012;2012:701976.
41. Tsybikov NN, Fefelova EV, Tereshkov PP, et al. Endothelial dysfunction in experi-mental hyperhomocysteinemia. Patol Fiziol Eksp Ter 2016;60:42-46.
42. Brough D, Tyrrell PJ, Allan SM. Regulation of interleukin-1 in acute brain injury. Trends Pharmacol Sci 2011;32:617-622.
43. Tehranian R, Andell-Jonsson S, Beni SM, et al. Improved recovery and delayed cytokine induction after closed head injury in mice with central overexpression of the secreted isoform of the interleukin-1 receptor antagonist. J Neurotrauma 2002;19:939-951.
44. Rothwell N. Interleukin-1 and neuronal injury:mechanisms, modification, and therapeutic potential. Brain Behav Immun 2003;17:152-157.
45. Zhang ML, Hou YC, Shen YL, et al. Probucol reverses homocysteine induced inflammatory monocytes differentiation and oxidative stress. European Journal of Pharmacology 2018;818:67-73.
46. Jiao JL, Qian L, Hua PD, et al. Homocysteine triggers inflammatory responses in macrophages through inhibiting CSE-H2S signaling via DNA hypermethylation of CSE promoter. Int J Mol Sci 2015;16:12560-12577. https://doi.org/10.3390/ijms160612560.
47. Chen Y, Liu H, Wang X, et al. Homocysteine up-regulates endothelin type A receptor in vascular smooth muscle cells through Sirt1/ERK1/2 signaling pathway. Microvasc Res 2017;114:34-40.
48. Rudd Jh, Hyafil F, Fayad ZA. Inflammation imaging in atherosclerosis. Arterioscler Thromb Vasc Biol 2009;29:1009-1016 https://doi.org/10.1161/ATVBAHA.108.165563.