



# Hyperhomocysteinemia as a Cause of Prevalent Intracerebral Small Vessels' Disease

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## Abstract

High homocysteine (HHcy) serum levels are a risk factor for small vessels' disease (SVD) rather than large vessels' disease (LVD), acting via endothelial dysfunction. The most common findings of cerebral SVD are: lacunar infarcts, white matter hyper density (leukoaraiosis) and micro bleeds. Clinical manifestations of these cerebral lesions are different, oscillating from the absence of any symptom to physical and behavioral, pathological manifestations, such as cognitive impairment, confusion or dementia, depression or disinhibition, difficulties of motility, etc. Therapeutically, intravenous thrombolytic treatment must be cautiously performed in cerebral SVD for secondary prevention. In addition, dual antiplatelet treatment and antihypertensive therapy should be cautiously used for primary prevention, to avoid episodes of intracerebral hemorrhage. On the contrary, folic acid and vitamins B6-12 supplementation appears to be useful to reduce severity and disability of both LVD and SVD. Concomitantly, statins and other anti-dysmetabolic drugs must be also employed to antagonize other possible causes of atherosclerosis.

**Keywords:** Homocysteine; Large vessels' disease (LVD) and small vessels' disease (SVD); Lacunar infarcts; Leukoaraiosis; Microbleeds

## Abbreviations

AIS: Acute Ischemic Stroke; HHcy: High Homocysteine; LVD: Large Vessel Disease; SVD: Small Vessel Disease; NO: Nitric Oxide; ROS: Reactive Oxygen Species; DNA: Desoxy-Nucleic Acid; NASCET: North American Symptomatic Carotid Endarterectomy Trial; SMART-MR: Second Manifestation of ARterial disease-Magnetic Resonance; ADMA: Asymmetric-Dimethyl-Arginine; BBB: Blood-Brain-Barrier; NINDS: National Institute of Neurological Disorders and Stroke; tPA: tissue-Plasminogen Activator; HOPE 2: Heart Outcomes Prevention Evaluation 2; VITATOPS-MR: VITamin To Prevent Stroke-Magnetic Resonance; SPARCL: Stroke Prevention by Aggressive Reduction in Cholesterol Levels; CT: Contrast Tomography; MRI: Magnetic Resonance Imaging.

## Introduction

Ischemic stroke subtypes (ISS) is a global health problem and one of the leading causes of long-term disability and death worldwide [1]. The site of vessels involved can be intracranial and extracranial, according to its position inside or outside the skull. Extracranial arterial vessels are large. On the contrary, intracranial vessels can be large or small (>/<200µm of diameter). Extracranial large arteries include: aortic arch,

common carotid artery, extracranial tracts of internal carotid artery and of vertebro-basilar artery. Intracranial large arteries are: carotid terminus, anterior and middle cerebral arteries, basilar artery and intracranial tract of vertebral arteries. But, intracranial small vessels also comprise: perforating cerebral arteries, arterioles, capillaries, and venules [2-5]. Trial of ORG 10172 Acute Stroke Treatment (TOAST) classified ischemic strokes into five groups, according to the presumed etiological mechanisms: large artery atherosclerosis, small vessel disease, cardio embolic disease, other strokes of determined etiology, and others of undetermined etiology [2]. A retrospective study of Wong demonstrated that extracranial large vessel disease is a more common cerebral lesion in Europe and in America, while intracranial vessel disease is more common cerebral lesion in Asian patients [6]. The majority of cerebral arterial disease is caused by chronic systemic hypertension, diabetes, dyslipidemia, smoking, advanced age, obesity, physical inactivity and others [7]. But, numerous observations refer a positive association between HHcy and ISS of large or small cerebral vessels' disease [8,9], with prevalent involvement of small vessels, as perforating arteries, arterioles, capillaries and venules [10-13].

## Homocysteine

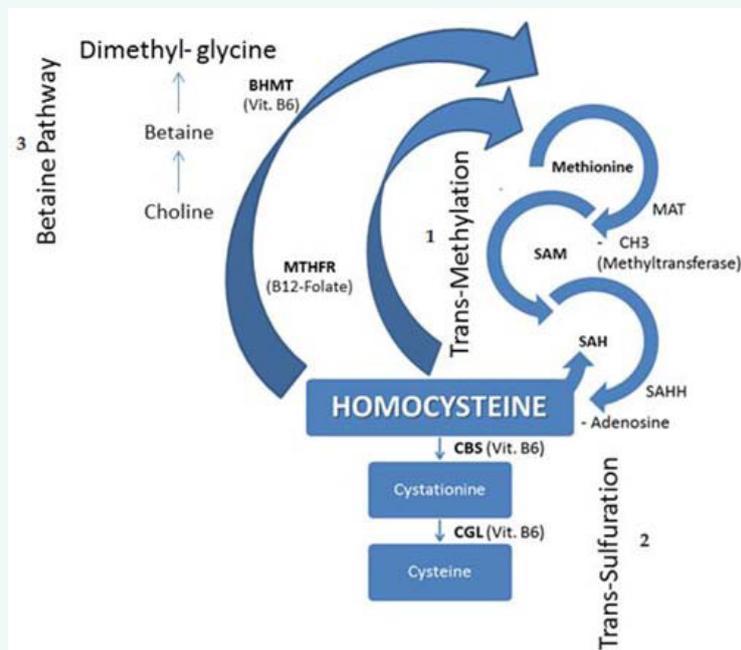
Homocysteine (Hcy) is a sulfur-containing amino acid produced from metabolism of the essential amino-acid, Methionine. It is further metabolized through two pathways: re-methylation and trans-sulfuration. The enzyme methionine synthase (MS) having vitamin B<sub>12</sub> as cofactor, remethylates Hcy back to Methionine. In this reaction, folate acts as a cofactor of the enzyme 5, 10-methylenetetrahydrofolate reductase (MTHFR). On the contrary, the enzyme cystathionine beta synthase (CBS) is required for the reaction of transsulfuration. This enzyme has vitamin B<sub>6</sub> as coenzyme. A third route of metabolization, is the Betaine-pathway only happening in liver and kidney (Figure 1). Abnormalities of one of three pathways lead to HHcy [14]. This condition depends on genetic and/or acquired defects. Genetic defects cause a reduction in activity of the enzymes involved in

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**Figure 1** Homocysteine Metabolism: three pathways. MTHFR: Methylen-Tetra-Hydro-Folate-Reductase; MAT: Methionine-Adenosyl-Transferase; SAM: S-Adenosyl-Methionine; SAH: S-Adenosyl-Homocysteine; SAHH: SAH-Hydrolyase; CBS: Cystationine-Beta-Synthase; CGL: Cystationine-Gamma-Lyase; BHMT: Betaine-Homocysteine-Methyl-Transferase.

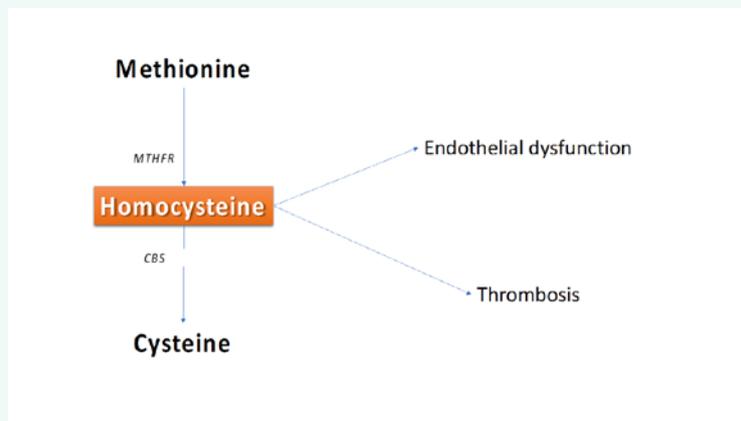
the remethylation pathway, such as MTHFR or MS. Causes of acquired conditions include vitamin deficiencies (particularly vitamin B<sub>6-12</sub> or folate), as well as renal impairment.

HHcy is recognized such as independent risk factor for coronary, cerebral and peripheral endothelial dysfunction and atherosclerosis [15,16]. The connection between HHcy and vascular lesions was firstly described by McCully [17]. Several mechanisms were proposed for the vascular lesions. These include oxidative stress, with increased production of Reactive Oxygen Species (ROS), cause of endothelial dysfunction. Pro inflammatory effects, suppressive growth of endothelial cells and vascular smooth muscle cells are also included [18,19]. Thrombosis activation via tissue factor expression, enhanced platelet reactivity, augmented factor V activity, and increased generation of thrombin can be present too [20-23]. HHcy can be associated with other already remembered pathologies or behavioral conditions, such as systemic hypertension, diabetes mellitus, smoking, physical inactivity or dyslipidemia, etc. This association may induce both LVD and SVD [24,25]. Previous reports proved that systemic hypertension, associated with HHcy, is preferably related to SVD [26]. On the contrary, diabetes, smoking or dyslipidemia, added to HHcy, are more frequently associated with LVD [27]. But, HHcy alone is more frequently related with cerebral SDV. The mechanisms responsible of this behavior are unknown too. Nevertheless, a major cause seems to be endothelial dysfunction, which is related to a decrease bioavailability of endothelium-derive nitic oxide (NO) and, subsequently inhibited vasodilation and increase thrombosis. HHcy also promotes arteriolar hypertrophy, resulting in occlusion of cerebral small arterioles [28-30]. Another mechanism specific

for degenerative brain disease, type Alzheimer's disease, is the action of endoplasmic reticulum stress-inducible protein (HERP) in enhance presenilin-mediated generation of amyloid beta-protein [31]. Intracranial small vessels' atherosclerosis mainly involves perforating cerebral arteries, arterioles, capillaries and venules [32]. In the SMART-MR (Second Manifestations of ARterial disease-Magnetic Resonance) studies was affirmed that cognitive decline and brain atrophy, frequently seen in older patients suffering from HHcy must be reported to hyperintensity of subcortical white matter and to lacunar or microbleeds lesions [33,34].

### Pathogenesis of SVD

As previously affirmed, endothelial dysfunction plays a pivotal role in intracerebral SVD [35,36]. Concordantly, it has been observed that reversal endothelial dysfunction obtained with antioxidant supplementation (as vitamin C, vitamin E, alpha-lipoic acid, etc.) reduces these lesions [37]. Asymmetric-D-Methyl-Arginine (ADMA), an endogenous inhibitor of endothelial NO synthase, plays a causal role in endothelial dysfunction, and is correlated with SVD severity [37-39]. In detail, the inhibition of NO synthase is responsible for impaired endothelium-dependent vasodilation, favors platelet hyperaggregability, and enhances monocyte adhesion [40]. In addition, the inhibition of this synthase induces vascular smooth cells proliferation and may contribute to the thickening of medial layer and/or myo-intimal hyperplasia [41]. Therefore, the clean effect deriving by the endothelial dysfunction consists in an increased arterial stiffness and decreased cerebral blood flow, responsible for following, overt atherosclerosis [42]. As consequences of these vascular,



**Figure 2** Two leading negative effects caused by homocysteine on arteries.

morphologic changes, the most frequent clinical manifestations of SVD are: cognitive impairment, mental confusion, imbalance of coordination, depression of mood, Alzheimer’s or Binswanger’s diseases, gait disturbances, severe headache and others frequent symptoms, as reported in Table 1 [43-45]. These clinical manifestations are prevalent. But, the most common morpho-functional findings found in aged patients suffering from HHcy are the major cause of SVD, i.e.: lacunar infarcts, white matter hyperdensity, and microbleeds.

### Lacunar infarcts

Lacunar infarcts refer to a small subcortical ischemia, resulting from the occlusion of perforating arterioles. The primitive lesion often is a small cavity, which represents an initial stage of lacunar infarct. But, some lacunes represent the sequences of previous larger infarct. Blockage of the orifice of branch arteries by a plaque in a parent artery also causes ischemia, often resulting in infarct. In some regions, the arteries were replaced by whols, tangles and wisps of connective tissue that obliterate the usual vascular layers. The media often is hypertrophied and contains fibrinoid material. The causes of these lesions are: thickening of the arterial media, with encroachment on the arterial lumen, and/or obstruction at origin of penetrating arteries, deriving by large intracranial artery intima plaques [46].

**Table 1:** The main symptoms depending from intracerebral SVD.

Leading symptoms of intra-cerebral SVD
-Cognitive impairment;
-Difficulties of motility;
-confusion
-imbalance of coordination
-Possibility of vascular dementia (Binswanger’s disease) or other dementias (Alzheimer’s disease)
Numbness
Weakness dizziness
-Depression of mood.

### White matter hyperintensity

Also called as leukoaraiosis, white matter hyperintensities are areas of sub-cortical white matter of demyelination as well as axonal and oligodendrocyte loss. Its pathogenesis is still incompletely understood. But, the hypothesis of ischemia and malfunctioning Blood-Brain-Barrier (BBB) seems to be effective, even if the primary event could be endothelial dysfunction [47]. The main cause inducing leukoaraiosis includes chronic ischemia secondary to atherosclerosis. Other frequent conditions are a fluctuation between hypotensive and hypertensive episodes. An association between cholesterol level and statin use is less consistent, even through this hypothesis was formulated. Low vitamin B<sub>12</sub> and folic acid can be also present. Clinically, at the initial stages, leukoaraiosis doesn’t have remarkable symptoms. On the contrary, in more advanced stages it may cause headache, tinnitus and dizziness. Problems in balancing, thinking and memorizing can be also present [48-50].

### Microbleeds

Cerebral microbleeds are small perivascular hemosiderin deposits (small chronic hemorrhages). The leading causes of these lesions are: systemic hypertension, diabetes, amyloid vasculopathy, elevated Hcy plasma levels, advanced age, low total serum cholesterol, chronic anti-thrombotic therapy, and others. They may be an indicator of increased risk of future hemorrhage [51]. It can associate with cognitive decline, gait disturbances, symptoms of depression or disinhibition.

### Therapeutic Measures

The National Institute of Neurological Disorders and Stroke (NINDS) trial is a double-blind study that evaluated the effect of intravenous recombinant tissue Plasminogen Activator (t-PA) in acute cerebral ischemic stroke by SVD. This treatment performed within three hours of the onset of symptoms for secondary prevention of ischemic event significantly improved clinical outcome at three months. Nevertheless, the use of intravenous t-PA should be cautious in SVD-patients, especially in those with multiple cerebral microbleeds, for increased risk of hemorrhage [52]. Contrarily to thrombolysis, the benefits of



antiplatelet drugs are considered similar for both primary and secondary prevention in lacunar or non-lacunar infarction, but not in cardioembolic stroke [53]. However, the incidence of major hemorrhage induced by dual antiplatelet therapy (aspirin + clopidogrel) was higher than that induced by aspirin alone [54]. Particularly, dual antiplatelet therapy should be avoided because the excessive risk of intracerebral hemorrhage [55,56]. In addition, in patients with ISS, the Heart Outcomes Prevention Evaluation-2(HOPE 2) study highlighted that lowering Hcy levels with folic acid and vitamins B<sub>6</sub>, B<sub>12</sub>, even through reduces the overall stroke risk, doesn't decrease stroke severity or disability. It must be added that the positive impact of vitamins' therapy on cerebral ischemia risk was most pronounced among subgroup in HHcy-younger than 70 years, and in patients not receiving folic acid fortification [57]. With reference to intracerebral SVD, the VITamin To Prevent Stroke-Magnetic Resonance Imaging (VITATOPS-MRI) sub-study evidenced that the employment of B vitamins is associated with a reduced white matter hyperintensity and lacunar infarctions [58]. In addition, vitamin E tocotrienols were recently found to attenuate the progression of white matter hyperintensity [59]. Therapeutic interventions against other risk factors for atherosclerosis (when are contemporary present) are requested. Among these, high blood pressure is a common risk factor. A value <130 mmHg of systolic blood pressure appears to be appropriated in HHcy-patients with lacunar infarction or matter hyperintensity. But, the long-term of blood pressure reduction remain uncertain, especially in old patients. Perhaps, that is related to the reduction of cerebral perfusion, due to a decreased autoregulatory mechanism [60]. Elevated LDL-cholesterol levels play an important role in the development of cerebral atherosclerosis. Thus, high doses of statins in these patients are able to significantly avoid secondary cerebral ischemia. But, its efficacy has been questioned in cerebral SVD for an increased bleeding risk of bleeding [61,62].

Other potential therapeutic strategies include steroid and non-steroid anti-inflammatory drugs. Pioglitazone, a PPAR-gamma inhibitor, has multiple properties that may attenuate cerebral SVD, including blood pressure modulation, anti-vascular inflammation, pro-endothelial activity and anti-fibrinolytic activity [63]. Lifestyle modifications must be also added. These include: smoking cessation, salt reduction, healthy diet and physical exercise. Finally, the prognosis of cerebral SVD is better. Than the other strokes (cerebral LVS) in the short term after onset, because the primary lesion is small [64].

## Conclusive Remarks

An increased Hcy levels is a risk factor for both LVD and SVD. The deleterious effects of HHcy are likely mediated by pro-atherogenic and pro-thrombotic effects (Figure 2). The first mainly contributes to SVD. On the contrary, prothrombotic effect is a leading cause of LVD [65]. Prothrombotic effect comes true via several mechanisms, such as tissue factor expression, attenuated anticoagulant processes, enhanced platelet reactivity, increased thrombin generation, augmented factor V activity, impaired fibrinolytic potential and vascular injury, including endothelial dysfunction [66]. On the contrary, pro-atherogenic effect mainly acts via endothelial dysfunction and ADMA plays a

major role in this dysfunction [3,36]. This causes an inhibition of NO synthase, of endothelin II and angiotensin II, of thromboxane A<sub>2</sub> with accumulation of prostaglandin H<sub>2</sub>. That is responsible for prevalence of vasoconstriction on vasodilation, representing the beginning of the atherosclerosis [67,68]. In turn, this condition favors the formation of lacunar infarcts, leukoaraiosis and microbleeds (ISS). Clinically, these lesions can fluctuate among the absence of any symptom ("silent" disease) to some, several signs and symptoms. They are rather frequent in aging population and represent, at moment, a major worldwide problem of public health, both in the presence and not of HHcy. Future studies performed in a wide range, are requested to evaluate the causality and the specific mechanisms by HHcy induce these manifestations, and to define the possible therapeutic effect of HHcy-lowering therapy when an increased Hcy plasma level presumably plays a major role.

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