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The link between homocysteine, folic acid and vitamin B12 in chronic kidney disease

In Depth Review

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ABSTRACT

Patients with chronic kidney disease or end-stage renal disease experience tremendous cardiovascular risk. Cardiovascular events are the leading causes of death in these patient populations, thus the interest in non-traditional risk factors such as hyperhomocysteinemia, folic acid and vitamin B12 metabolism is growing. Hyperhomocysteinemia is commonly found in CKD patients because of impaired renal metabolism and reduced renal excretion. Folic acid, the synthetic form of vitamin B9, is critical in the conversion of homocysteine to methionine like vitamin B12. Folic acid has also been shown to improve endothelial function without lowering homocysteine, suggesting an alternative explanation for the effect of folic acid on endothelial function. Whether hyperhomocysteinemia represents a reliable marker of cardiovascular risk and cardiovascular mortality or a therapeutic target in this population remains unclear. However, it is reasonable to consider folic acid with or without methylcobalamin supplementation as appropriate adjunctive therapy in patients with CKD. The purpose of this review is to summarize the characteristics of homocysteine, folic acid, and vitamin B12 metabolism, the mechanism of vascular damage, and the outcome of vitamin supplementation on hyperhomocysteinemia in patients with CKD, ESRD, dialysis treatment, and in kidney transplant recipients.

KEYWORDS: hyperhomocysteinemia, folic acid, vitamin B12, chronic kidney disease, end-stage renal disease, cardiovascular disease

Introduction

Chronic Kidney Disease (CKD) represents an important economic burden for health systems around the world, with an estimated global prevalence of between 11 and 13%. Rationalized measures are needed to slow the progression to end-stage kidney disease (ESRD) and to decrease cardiovascular mortality [1]. Mortality rates remain in fact above 20% per year with the use of dialysis, with more than half of all deaths related to cardiovascular disease [2]. The problem of peripheral arteries disease (PAD) is also emerging, which is more common in patients with CKD and is associated with lower limb amputations and increased mortality [3].

Traditional factors such as hypertension, dyslipidaemia and diabetes mellitus are not sufficient to explain the dramatically increased cardiovascular risk in the population with CKD/ESRD. Thus, much attention shifted to other less studied aspects of CKD such as oxidative stress, endothelial dysfunction, chronic inflammation, vascular calcification in chronic kidney disease-mineral and bone disorder (CKD-MBD) and finally hyperhomocysteinemia (HHcy) [4].

The latter, since its discovery, proved to be a plausible risk factor for the development of atherosclerotic vascular disease processes leading to cardiovascular disease (CVD) and stroke. Levels of homocysteine (Hcy) higher than 20.0 µmol/L are associated with mortality 4.5 times higher. The "homocysteine hypothesis" is supported by the fact that subjects with problems in the enzymatic pathway of homocysteine metabolism have a higher level of homocysteine than the general population and a faster progression of arteriosclerosis. Therefore, the link between cardiovascular mortality and arteriosclerosis has been the subject of debate with conflicting results [5].

The high prevalence of HHcy in patients with CKD generated interest in a potential role of HHcy as a risk factor for CKD progression and CVD [5,8,9,10].

Hcy is a non-essential, sulfur-containing, non-proteinogenic amino acid, synthetized by transmethylation of the essential, diet-derived amino acid methionine (Figure 1). Aberrant Hcy metabolism could lead to redox imbalance and oxidative stress resulting in elevated protein, nucleic acid and carbohydrate oxidation and lipoperoxidation, products known to be involved in cytotoxicity [11].

Hcy levels can be significantly reduced by supplementation with folic acid (FA), vitamin B12 and vitamin B6. However, in several randomized and controlled studies the impact of vitamin supplementation seems to be disappointing in terms of cardiovascular mortality [6,7]. The debate is still open: some studies have reported a null or harmful effect of supplementation with FA and B vitamins, including cyanocobalamin [10], while others have confirmed a link between the homeostasis of the vitamins, cardiovascular risk and CKD progression [12]. These two outcomes are ultimately considered the result of a complex interaction between the effects of HHcy, FA, enzymatic activity/gene variants, and FA fortification programs that exist in some countries [13].

B vitamins and homocysteine metabolism

Folic acid/Vitamin B9

The term "folate" includes several forms of vitamin B9, including tetrahydrofolic acid (the active form), methyltetrahydrofolate (the primary circulating form), methenyltetrahydrofolate, folinic acid, folacin and pteroylglutamic acid. Since the human body is not able to synthesize folate, it must be provided through the diet [14]. Folic acid comes from polyglutamates that are converted into monoglutamates in the intestine, and then transported through mucous epithelium by a specific vector [15].

Cobalamin/Vitamin B12

Vitamin B12, also known as cobalamin, is a nutrient with a key role in human health: it is essential as a cofactor for the enzyme methionine synthase and other biochemical reactions, such as beta oxidation of fatty acids or DNA synthesis, and in the production of red blood cells [17–18]. Vitamin B12 deficiency is a common cause of HHcy and a frequent feature of patients with CKD [14–16].

Cobalamin is one of the most complex coenzymes in nature. The molecule consists of a corrinic ring and a part of dimethylbenzimidazole (DMB), and the focal point of the structure is the cobalt atom, held in the center of the corrinic ring which bonds some chemical groups, the most important of which are the hydroxyl group (hydroxocobalamin, OHCbl) and group CN (cyanocobalamin, CNCbl). These are the forms most commonly used in pharmaceutical formulations for vitamin B12 supplementation.

Vitamin B12, when ingested, is complexed with salivary haptocorrin, and cobalamin is released from pancreatic proteases in the duodenum. Then, cobalamin binds to an intrinsic factor secreted by the parietal cells of the stomach: when this complex reaches the distal ileum, it is endocytosed by enterocytes through cubilin. Then, it is transported into the plasma by a plasma transport protein called transcobalamin. B12 is filtered by the glomerulus; however, urinary excretion is minimal under normal conditions, due to reabsorption in the proximal tubule [19].

Metabolism of homocysteine and folate cycle

As mentioned above, Hcy plasma levels are determined by several factors, such as genetic alterations of the methionine metabolism enzymes, and vitamin B12, vitamin B6 and folic acid deficiency. FA, playing a pivotal role in Hcy metabolism, is inert and requires to be activated in tetrahydrofolic acid, a precursor of 5-methyltetrahydrofolate (5-MTHF). Methylenetetrahydrofolate reductase (MTHFR) is a key regulatory enzyme involved in folate dependent Hcy remethylation. MTHFR catalyzes the reduction of 5,10-methyltetrahydrofolate to 5-MTHF, necessary for the normal activity of the enzyme methionine synthetase (MTS), which uses vitamin B12 as a cofactor and converts homocysteine into methionine [20]. Methionine is transformed into S-adenosylmethionine (SAM) and then converted to S-adenosylhomocysteine (SAH) through a reaction catalyzed by methionine synthase reductase (MTRR). SAM is one of the most important donors of methyl groups and is fundamental in the catabolism of various amino acids and fatty acids [21].

Hcy is the final product, derived from the hydrolysis of SAH to Hcy and adenosine, and is located at the center of two metabolic pathways: it is irreversibly degraded through the path of transsulfuration into cysteine or is remethylated to methionine (folate cycle).

1. ***Transsulfuration***: Firstly, Hcy combines with serine by forming cystathionine via cystathionine-beta-synthase (CBS); then, cystathionine is hydrolyzed into cysteine and alpha-ketobutyrrate from cystathionine-gamma-lyase (CTH). Human CBS is expressed in the liver, kidneys, brain and ovaries and, during the first embryogenesis, in the neural and cardiac systems.
2. ***Remethylation***: Hcy conversion into methionine is catalyzed by the enzyme MTS and connects the cycle of folates with Hcy metabolism. While the MTS enzyme is expressed ubiquitously, another Hcy remethylation system, betaine-Hcy methyltransferase, is expressed mainly in the liver and kidneys [1].

The main reactions of Hcy metabolism are summarized in Figure 1.

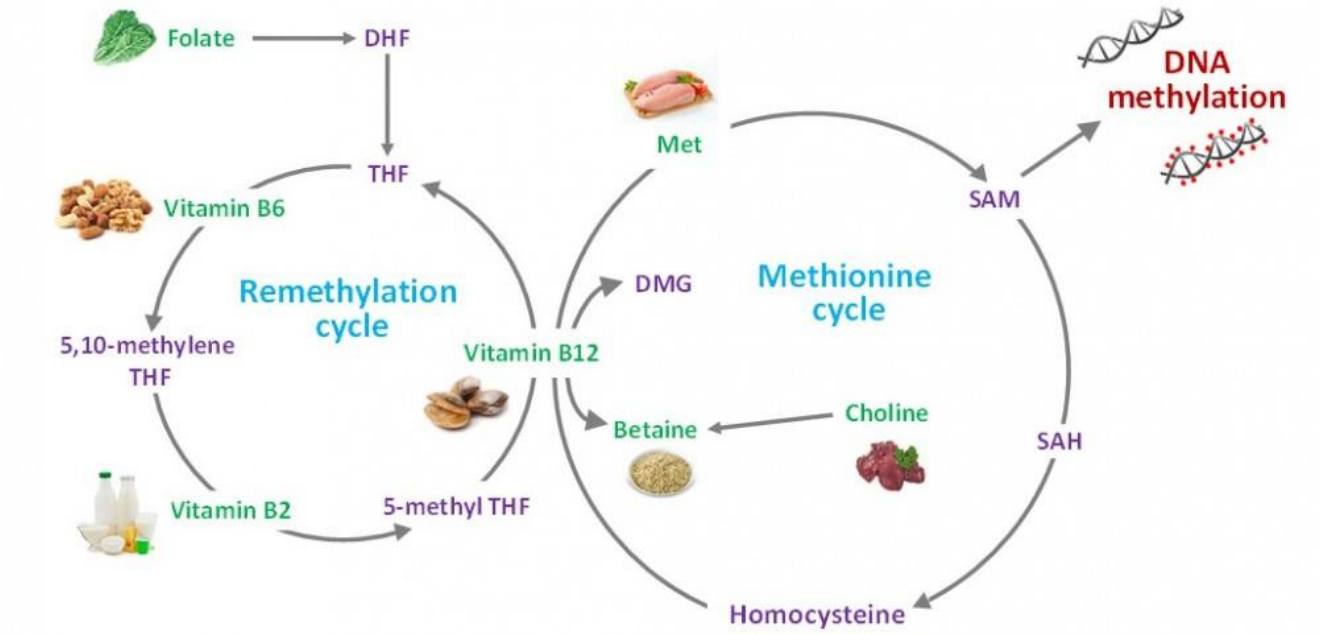

Figure 1: Schematic representation of homocysteine metabolic pathway. DHF: dihydrofolate; DMG: N,N-dimethylglycine betaine; Met: methionine; SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine; THF: tetrahydrofolate

Folic acid metabolism, vitamin B12 and homocysteine in CKD

Homocysteine

Patients with CKD and ESRD have been shown to have higher blood levels of Hcy than the general population [22]. The normal plasma level is <10 µmol/L; levels of Hcy <16 µmol/L are defined as mild HHcy, while severe HHcy is diagnosed when the levels are >100 µmol/L [23]. About 80-90% of the circulating Hcy is protein-bound; 10-20% of total homocysteine (tHcy) is present as Hcy-cysteine and Hcy mixed disulfide (Hcy dimer), and <1% is present in the reduced free form [14]. In CKD, studies show that the cause of HHcy is a reduced clearance rather than an increase in production, but the exact site of altered clearance remains controversial: under physiological conditions, only non-protein related Hcy is subjected to glomerular filtration and is then mostly reabsorbed into the tubules and oxidized into carbon dioxide and sulfate in kidney cells [24]. Some data support the hypothesis that decreased Hcy removal in CKD is caused by a decreased intrarenal metabolism, through both transsulfuration and remethylation [25].

Folic acid

It has also been shown that an anionic inhibition of the membrane transport of 5-MTHF occurs in patients with CKD with a depression in the intracellular incorporation rate of folates. These results suggest that the level of folates measured in the blood of uremic individuals does not reflect its intracellular use because the uptake is altered due to anionic inhibition [26].

Vitamin B12

Mainly linked to proteins in the blood, about 20% of circulating B12 is related to holotranscobalamin (TC2). The kidney plays an important role in TC2 metabolism, as TC2 is filtered into the glomerulus and is reabsorbed into the proximal tubule. Defects in protein resorption in the proximal tubule could therefore lead to a biologically active loss of CT2 in the urine. Increased levels of TC2 were observed in patients with CKD. Despite this, there is a decrease in TC2 absorption in cells that can lead to a paradoxical increase in cell Hcy levels, despite normal total B12. Thus, a functional

deficiency of B12 can occur in patients with CKD as part of an increase in TC2 leaks in the urine, lower absorption of CT2 in the proximal tubule, and lower cellular absorption of TC2.

It is also important to consider that high levels of B12 could be harmful to individuals with CKD. This is related to cyanide metabolism, which is abnormal in individuals with CKD due to the decreased glomerular filtrate. Cyanocobalamin, the most common form of B12 replacement, is metabolized into active methylcobalamin, releasing small amounts of cyanide. Under normal circumstances, methylcobalamin binds to cyanide converting it to cyanocobalamin. However, in patients with CKD, reduced cyanide clearance prevents the conversion of cyanocobalamin into the active form, and therefore integration into this form is less effective in reducing Hcy levels. In addition, the excessive amount of supplementation with cyanocobalamin can release cyanide ions that are not excreted and contribute to the onset of complications in the patient with CKD (e.g. uremic neuropathy) [27–28].

Methylenetetrahydrofolate reductase polymorphisms

MTHFR plays a key role in Hcy metabolism and catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyl-THF, the predominant circulating form of folate [29]. The MTHFR gene encodes the enzyme methyltetrahydrofolate reductase and is localized on chromosome 1 (1p36.3). Genetic polymorphisms involved in the homocysteine-methyonine route have been shown to result in HHcy. Although several MTHFR gene variants have been identified, the most characterized are single nucleotide polymorphisms (SNPs) in position 677 (MTHFR 677C>T), in position 1298 (MTHFR 129 8A>C), in position 1317 (MTHFR 1317T>C) and in position 1793 (MTHFR 1793G>A). It has been proposed that the two common mutations, MTHFR C677T and A1298C, may be associated with congenital abnormalities, cardiovascular diseases, strokes, cancer and clotting abnormalities [30,31].

C677T polymorphism is characterized by a point mutation at position 677 of the MTHFR gene that converts a cytosine into a thymine. It is known that when alanine replaces valine in the enzyme at the folate binding site, this polymorphism is commonly called thermolabile, because the activity of the encoded enzyme is reduced by 50-60% at 37°C and by 65% at 46°C. People who are homozygous for C677T tend to have slightly increased blood Hcy levels if their folate intake is insufficient, but normal Hcy levels if folate intake is adequate [32]. Substitution 677C>T is the most common missense variation of MTHFR, with a global prevalence of 40%. The frequency of C677T homozygosis varies depending on the ethnicity: from 1% or less among blacks in Africa and the United States, to 25.3% or more among Italians, Hispanic Americans and Colombians [30]. In contrast, the frequency of the mutant T allele in the MTHFR C677T gene in the Chinese population is 41.7%, higher than in other populations and could be an independent risk factors of early renal damage in the hypertensive Chinese population [33].

A1298C polymorphism is characterized by a point mutation in position 1298 in exon 7 of the MTHFR gene responsible for an amino acid substitution of a glutamine with an alanine in the enzyme regulatory domain. The activity of the encoded enzyme decreases, but to a lesser extent than in the case of C677T polymorphism. Subjects who are homozygotes for the A1298C allele do not appear to have increased serum Hcy levels [30,31]. According to Trovato et al., MTHFR 677C>T and A1298A>C gene polymorphisms could have a protective role on renal function as suggested by the lower frequency of both polymorphisms among a population of 630 dialysis patients in end-stage renal failure [34]. Regarding the other most common SNPs, MTHFR 1317T>C is a silent mutation, while MTHFR 1793G>A results in amino acid replacement, but with no impact on the functional activity of the enzyme [31].

The link between Hcy level and MTHFR gene polymorphisms has been investigated by Malinow et al.: homozygote subjects for the MTHFR T677 allele have shown an important reduction in the plasma levels of tHcy after FA integration. On the other hand, C677 allele homozygosity, especially subjects with higher basal folate levels, have shown a lesser tHcy reduction after FA supplementation. Finally, the carriers of the T/T genotype have shown the sharpest decrease of tHcy with FA integration [35]. This result was confirmed by Anchour et al: the simultaneous supplementation of folate and vitamin B12 was only useful in the homozygotes for the C allele and the reduction of Hcy was significantly higher in the carriers of the TT genotype than in other genotypes (CC/CT) [36]. These findings are consistent with the China Stroke Primary Prevention Trial (CSPPT), in which the largest decrease in serum Hcy was seen in the carriers of the TT genotype [37]. The relationship between MTHFR polymorphism and coronary heart disease severity showed that Hcy levels were significantly higher in patients with coronary arteries disease (CAD) than in control subjects and the genotype of MTHFR 677C>T was associated with increased CAD severity in patients at high risk for this pathology [38]. In summary, most available evidence suggests that MTHFR polymorphisms may influence folic acid and vitamin B12 treatment response in terms of Hcy lowering and cardiovascular risk reduction in patients with CKD and ESRD although indication of routine testing is matter of debate [39].

Endothelial damage of homocysteine and impact of CVD in ESRD patients

The pathogenic role of HHcy on the cardiovascular system in CKD and ESRD is related to the progression of atherosclerosis in the context of an already increased risk of vascular damage caused by the uremic syndrome. The mechanisms by which endothelial damage occurs are (Figure 2):

- *Oxidative stress.* HHcy helps generate reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive species of thiol, thus decreasing the bioavailability of nitrogen monoxide (NO). These processes trigger latent matrix-metalloproteinase (MMP) and make the tissue inhibitor of metalloproteinase (TIMP) inactive. This leads to adverse cardiovascular remodelling, with increased collagen deposit [40]. HHcy significantly reduces the expression of the endothelial synthase nitric oxide protein (eNOS) in a dose-dependent manner and ultimately causes impaired basal production of NO, formation of radicals and subsequent endothelial damage by decreasing the bioavailability and bioactivity of NO [41].
- *Inflammation.* Through the activation of the nuclear factor kappa B (NF-κB), a transcription factor known to stimulate the production of cytokines, chemokines, leukocyte adhesion molecules, HHcy induces the expression of proinflammatory chemokines MCP-1 and IL-8 in endothelial cells by enhancing transendothelial migration of monocytes, vascular inflammation and atherogenesis [42–43]. As for low-density lipoproteins (LDL), N-homocysteination produces aggregation, thus the accumulation of cholesterol, and facilitates the mediated absorption of oxidized LDL by macrophage scavenger receptors, resulting in the formation of foam cells in atherosclerosis [43–44].
- *Proliferation of smooth muscle cells.* HHcy can significantly promote vascular smooth muscle cells (VSMC) proliferation, by promoting the expression of adhesion molecules, chemokines and VSMC mitogen [45]. HHcy can act directly on glomerular cells by inducing sclerosis and trigger kidney damage by reducing the plasma and tissue level of adenosine. The decrease in plasma adenosine in turn leads to a greater proliferation of VSMC, accelerating the sclerotic process in the arteries and glomeruli. In a pattern of folate-free HHcy rat, glomerular sclerosis, mesangial expansion, podocyte dysfunction, and fibrosis all occurred due to increased local oxidative stress [46].

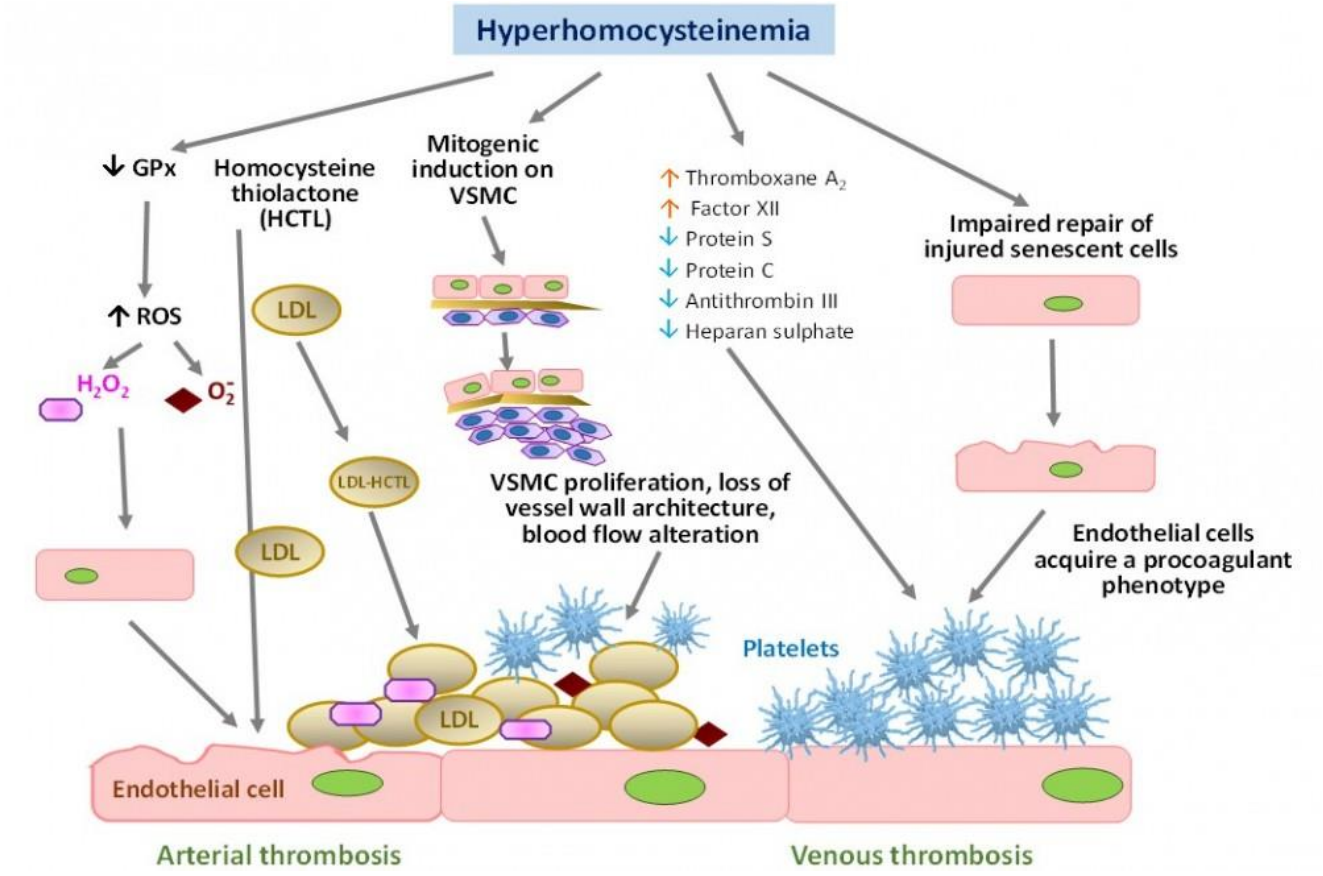

Figure 2: Main pathogenetic pathways of endothelial damage mediated by hyperhomocysteinemia

These pathways end up amplifying the atherosclerotic process and inflammatory state present in CKD [47]. For patients with CKD and ESRD, despite the increase in Hcy levels (average level of Hcy in the general population about 10-15 µmol/L versus 25-35 µmol/L in uremic patients), the role of Hcy as a cardiovascular risk and mortality factor is still uncertain and many retrospective and interventional studies have given rise to conflicting evidence [48].

Folic acid supplementation in patients suffering from CKD

There is a large body of evidence indicating that folate therapy improves HHcy in the general population, but the data is less clear in CKD and ERSD patients [39,49]. The main interventional studies on the use of folic acid and vitamin B12 in CKD patients are summarized in Table 1. The benefits of folate supplementation in subjects with reduced renal function do not seem to lie entirely in the lowering of serum Hcy. Endothelial dysfunction is a key process in atherosclerosis and independently predicts cardiovascular events. High-dose FA (5 mg per day), alone or in combination with other B vitamins, appears to improve endothelial function through a largely Hcy-independent mechanism [50]. Endothelial cells can be particularly vulnerable to HHcy, as they do not express CBS, the first enzyme of the transsulfuration pathway [51]. Therefore, endothelial cells can eliminate Hcy only through remethylation, and normal activity of the enzymatic route is thus essential to prevent the increase of Hcy to a pathological level [52]. FA improves endothelial function by reducing intravascular oxidative stress; also improves intracellular superoxide generation by increasing the half-life of NO [53]. Folate therapy reduces but does not normalizes Hcy levels, frequently elevated in CKD patients. The mechanisms of this folate resistance have not been fully elucidated, yet. The entry of folate into the cell is mediated by specific folate receptors, whose expression is also modulated by the folate state, through an Hcy-dependent regulation mechanism. In peripheral

mononuclear cells of hemodialysis patients, FR2 expression decreased and did not respond to changes in Hcy concentration [54].

Use of folate and vitamin B12 in the prevention of cardiovascular mortality and in slowing the progression of CKD

The role of folic acid and vitamin B12 supplementation in reducing mortality and preventing progression to ESRD is still to be determined. According to the meta-analysis of Heinz et al. of retrospective, prospective and observational studies on total 5123 patients, HHcy emerged as a risk factor for cardiovascular events and mortality in ESRD, especially in those subjects who do not receive additional FA (in countries without fortification programmes). Prospective studies have shown that in patients with ESRD, a 5 µmol/L increase in Hcy concentration is associated with a 7% increase in the risk of total mortality and a 9% increase in the risk of cardiovascular events. The level of Hcy in these patients seems to have decreased of 13 to 31 µmol/L due to supplementation with B vitamins in intervention studies. This was associated with a 27% reduction in the risk of cardiovascular events, although mortality had not decreased [55].

The minimum dose of folic acid to achieve a reduction of Hcy is debated: non-diabetic ESRD patients can respond to a daily dose of 5 mg FA, but diabetic patients with ESRD may need up to 15 mg to reduce the Hcy level more than 20% and have benefits on CVD risk, regardless of FA fortification. In addition, simultaneous administration with vitamin B12 is more effective in counteracting HHcy [56]. In non-diabetic patients with mild to moderate CKD a treatment strategy with pravastatin, vitamin E and Hcy reduction therapy (vitamin B12 and folate) leads to a significant reduction in the progression of carotid stenosis and a significant improvement in endothelial function and urinary excretion of albumin. However, no significant effect on the eGFR has been observed [57]. Similar results have emerged in the meta-analysis of Quin et al. including studies carried out from 1966 to 2011, for a total of 3886 patients with ESRD and CKD, where the relationship between supplementation with B12, FA and CVD had been analyzed after 24 months of treatment. FA therapy reduced the risk of CVD by 15%. Greater benefits were observed in those trials with a treatment duration >24 months, a decrease in Hcy level >20% ($P = 0.007$), and no or partial FA fortification ($P = 0.04$). The positive effect was seen when Hcy levels decreased >20%, even in the presence of FA fortification [58]. However, a reduction in Hcy secondary to high-dose FA therapy does not correspond to an increase in survival nor to a reduction of cardiovascular events according to randomized double-blind studies [59]. In the meta-analysis by Pan et al. (10 studies of patients in CKD), Hcy-lowering therapy is not associated with reduction of CVD, stroke and all-cause mortality [60]. However, the cohort of patients recruited had a high number of diabetic patients from areas with a grain fortification program.

Although HHcy is associated with increased CKD progression and albuminuria [61], the DIVINE study investigated the effects of Hcy-lowering therapy with high doses of folate (40 mg/day), vitamin B12 (1000 mg/day) and vitamin B6 (2 mg/day) in patients with diabetic nephropathy and showed that this treatment regimen does not increase survival or slow progression in ESRD, but rather leads to a higher incidence of cardiovascular events and a greater decrease in eGFR [62]. A possible explanation for these negative results can be attributed to the high load of cardiovascular comorbidity and to suboptimal therapy compliance. In addition, the study considered the CKD and ESRD population together and not separately. The above-mentioned China Stroke Primary Prevention Trial (CSPPT), a large, randomized study among adults with high blood pressure without a history of stroke or myocardial infarction, found that a therapy with ACE inhibitors and FA significantly reduced the relative risk of first stroke by 21%, more than ACE inhibitors alone. Among

individuals with MTHFR 677 CC or CT genotypes, those with the lowest basal folate levels have the highest risk of stroke and benefit the most from FA therapy. In addition, individuals with the TT genotype may require a higher dosage of FA to exceed biologically insufficient levels [37]. An exploratory analysis by subgroups to assess the effect of treatment on primary outcome in various subgroups of CKD participants showed that the reduction in the risk of CKD progression was more represented in the diabetes subgroup [63]. Of note, CSPPT study selected a population without fortification of cereals with folic acid.

Several factors including age, baseline Hcy levels, FA fortification of grains, B12 status, renal function, comorbidities, and medications could modify the effects of folic acid and vitamin B12 on cardiovascular risk. The available evidence regarding the effect of Hcy lowering therapies on CKD progression is controversial and further studies are needed, with CKD progression as primary endpoint and with a more homogeneous population selection [39].

The role of folate and vitamin B12 therapy

ESRD patients in chronic dialysis treatment

In many cases, the literature has shown that dialysis and ESRD patients are a peculiar population whose response to certain factors is opposite to that of the general population, a condition that has been called “reverse epidemiology” [64]. A curious example is hypocholesterolemia, identified as a predictor of higher mortality in dialysis patients [65]. Similarly, data from our group have previously shown that a higher BMI protects ESRD patients from coronary artery calcifications [66], in line with a meta-analysis by Lowrie et al, based on 43,334 hemodialysis patients, indicating an improved survival associated with increased BMI values [67].

In line with this theory, very low Hcy levels appear to be associated with worse clinical outcomes, longer hospitalization, and higher mortality from all causes, and cardiovascular mortality in ESRD patients [68]. The combined effect of protein-energy malnutrition and inflammation may partly explain the apparent paradox represented by the inverse relationship between Hcy level and mortality in patients with ESRD [14].

The study of Sohoo et al. examined a cohort of 12,968 hemodialysis patients treated with vitamin B12 for 5 years, to observe the relationship between serum folate/B12 and mortality. Concentrations of B12 ≥550 pg/mL are associated with increased mortality from all causes in hemodialysis patients, regardless of sociodemographic data and laboratory variables [12]. The effectiveness of high-dose folic acid in event prevention in ESRD was evaluated in a randomized study. A total of 510 patients on chronic dialysis were randomized to 1.5 or 15 mg of FA contained in a renal multivitamin with a median follow-up of 24 months. Composite mortality rates and cardiovascular events did not differ between the FA groups. High basal Hcy was associated with lower event rates, which would confirm an inverse relationship between Hcy and events in ESRD patients. The administration of FA at high doses did not affect event rates [69]. Similar studies have come to the same conclusion: the Atherosclerosis and Folic Acid Supplementation Trial (ASFAST) recruited a total of 315 subjects with chronic kidney failure (most of them in dialysis) who were randomized to 15 mg FA per day or placebo and followed for a median of 3.6 years. Total Hcy in plasma is reduced by 19% in the FA group but this does not slow down the progression of atherosclerosis nor improve morbidity or cardiovascular mortality in patients [57].

Supplementation with B vitamins along with FA could be an alternative in reducing vascular oxidative stress. However, the randomized multicenter study conducted in double-blind by Heinz et al. on 650 patients in hemodialysis undergoing supplementation with FA, vitamin B12 and vitamin B6, showed

that such therapies did not reduce total mortality and had no significant effect on the risk of cardiovascular events in patients with end-stage kidney disease [62]. Normalization of Hcy levels is difficult to achieve in dialysis patients with FA alone: according to Righetti et al., only 12% of a cohort of 81 patients in chronic dialysis has reached normal levels of Hcy. However, this condition has again shown no benefit in terms of survival [70].

The changes in the uremic patient's metabolism described in the previous sections leave an open question regarding FA and vitamin B12 supplementation in dialysis.

Another study by Righetti suggested that folate therapy to lower Hcy can reduce cardiovascular events in dialysis patients [71]. In a study by our group on a population of 341 patients in chronic dialysis, group A was treated with 50 mg i.v. of 5-MTHF, and group B was treated with 5 mg/d of oral FA. Both groups received vitamin B6 and B12.

Our data showed that I.V. 5-MTHF appears to improve survival in hemodialysis patients regardless of the lowering of Hcy [72]. This latest evidence confirms that the role of FA and vitamin B12 should be better understood in this category of patients, both at the biochemical level and at the level of clinical outcomes.

Study, year	Duration, design	Population	Treatment	Outcomes
Nanayakkara PW et al, 2007 [57]	2 yrs, double-blind RCT	93 patients with mild to moderate CKD	Pravastatin, vitamin E, and homocysteine lowering therapy (daily 5 mg FA + 100 mg vitamin B6 + 1 mg vitamin B12) vs placebo	In the treatment group significant reduction in CC-IMT, increase in BA-FMD, improvement in endothelial function and urinary albumin excretion, no effect on eGFR
Jamison RL et al, 2008 [58]	7 yrs, double-blind RCT	2056 patients with CKD (n=1305) or ESRD (n=751) and HHcy (≥ 15 mmol/L)	Daily 40 mg FA + 100 mg vitamin B6 + 2 mg vitamin B12 vs placebo	In the treatment group significant lowering of Hcy levels, no effect on secondary outcomes (MI, stroke, and amputations time to dialysis and mortality)
Zoungas S et al, 2006 [61]	3.6 yrs, double-blind RCT	315 patients with CKD	Daily 15 mg FA vs placebo	In the treatment group lowering by 19% of Hcy levels, no effect on secondary outcomes (change of IMT, artery function MI, stroke, cardiovascular death and overall cardiovascular events)
Heinz J et al, 2010 [62]	6 yrs, double-blind RCT	650 ESRD patients under hemodialysis treatment	5 mg FA + 50 mg vitamin B12 + 20 mg vitamin B6 (active treatment) vs or 0.2 mg FA, 4 mg vitamin B12 + 1.0 mg vitamin B6 (placebo) 3 times/week for 2 yrs	No effect on total mortality and fatal or nonfatal cardiovascular events
Xu X et al, 2016 [63]	4.5 yrs, double-blind RCT	1671 patients with CKD	Daily 10 mg enalapril + 0.8 mg FA (n=7545) vs 10 mg enalapril alone (n=7559)	In patients receiving enalapril + FA the risk for CKD progression and the rate of eGFR decline were decreased by 56% and 44%, respectively
Wrone EM et al, 2004 [63]	2 yrs, RCT	510 ESRD patients under hemodialysis treatment	Daily 1, 5, or 15 mg FA contained in a renal multivitamin	No effect of high-dose FA administration on the rates of cardiovascular events and mortality
Righetti M et al, 2003 [70]	1 yr, RCT	81 ESRD patients under hemodialysis treatment	Daily 15 mg FA (n=25) vs 5 mg FA (n=26) vs untreated (n=30)	No significant improvement of HHcy, regardless of FA dose, but treated patients tended towards a decreased rate of cardiovascular events.

Righetti M et al, 2006 [71]	871 days (median follow-up, range 132-1825 days), single-center, open, randomized prospective trial	114 ESRD patients under hemodialysis treatment	5 mg daily FA, or 5 mg every other day (if serum FA levels were up the normal high limit of 16.8 ng/mL) + vitamin B complex (250 mg B1 + 250 mg B6 + 500 mg B12, if plasma vitamin B12 values were below the normal limit of 200 ng/L)	Lower rate of cardiovascular events in treated patients with low Hcy levels
Cianciolo G et al, 2008 [72]	55 months, randomized prospective study	341 ESRD patients under hemodialysis treatment	Patients were randomized into two groups: group A (n=174) treated with I.V. 50 mg 5-MTHF (Prefolic) three times a week (end of each dialysis session) vs group B (n=167) treated with daily 5 mg FA. Both groups also received I.V. 300 mg vitamin + 1 g vitamin B12 at the end of the dialysis session.	Both FA acid and 5-MTHF decreased Hcy levels, and I.V. 5-MTHF improved survival in hemodialysis independent from Hcy lowering. CRP but not HHcy resulted to be the main risk factor for mortality in hemodialysis patients
Buccianti G et al, 2001 [74]	6 months, cross-sectional clinical study	55 ESRD patients under hemodialysis treatment	27 patients with macrocytosis treated the end of each dialysis session with I.V. 0.9 mg folinic acid + 0.5 mg cyanocobalamin + 1.5 mg hydroxycobalamin vs 28 untreated patients	Intermittent I.V. administration of folinic acid combined with vitamin B12 resulted in lower HHCy plasma concentration, but the effect was also related to genotype and dialysis modality
Bostom AG et al, 2011 [78]	5 yrs, multi-center double-blind RCT	4110 stable kidney transplant recipients	Participants were randomized to receive either a high dose (n=2056) of FA (5.0 mg), vitamin B6 (pyridoxine; 50 mg) and vitamin B12 (cyanocobalamin; 1.0 mg) or a low dose (n=2054) of vitamin B6 (1.4 mg) and vitamin B12 (2.0 µg) and no FA.	In the high dose treatment arm, a significant reduction in Hcy level was achieved, but without any beneficial impact on cardiovascular outcomes, all-cause mortality, or allograft failure

Table 1: Summary of major interventional studies on folic acid / vitamin B12 administration in patients with CKD

BA-FMD: brachial artery flow-mediated dilatation; CC-IMT: carotid intima-media thickness; CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate; ESRD: end-stage renal disease; FA: folic acid; HHcy: homocysteinemia; I.V.: intravenous; MI: myocardial infarction; RCT: randomized controlled trial; yr(s): year(s)

Role of FA and B12 supplementation in CKD anemia

In uremia-related anemia, unless patients with CKD and ESRD show significant folate depletion, additional FA supplementation does not appear to have a beneficial effect on erythropoiesis or response to recombinant human erythropoietin therapy (rHuEPO). However, measurements of folate circulating in the serum do not necessarily reflect folate reserves in tissues, and folate measurements in red blood cells provide a more accurate representation.

The low concentrations of folate in red blood cells in these patients suggest the need for FA supplement [73]. Megaloblastic anemia, that occurs in vitamin deficiencies frequently found in uremic patients, results from inhibition of DNA synthesis during the production of red blood cells [74]. When cobalamin levels become inadequate, DNA synthesis is compromised, and the cell cycle cannot progress from the G2 growth phase to the mitosis phase.

This leads to continuous cell growth without division, and then to macrocytosis [14]. In patients with CKD, folate and vitamin B12 deficiency may represent an important factor in renal anemia and hyporesponsiveness to rHuEPO therapy [75].

Kidney transplant recipients

In kidney transplants, several factors such as dialysis vintage, anemia, immunosuppression, inflammatory state, and dysmetabolic alterations can affect the cardiovascular risk [76,77]. The effect of supplementation of FA, vitamin B12 and vitamin B6 on CVD and mortality reduction has been studied by the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) study. Kidney transplant recipients were randomized to a daily multivitamin drug containing high doses of folate (5.0 mg), vitamin B12 (1.0 mg) and vitamin B6 (50 mg), or placebo. Despite the actual lowering the Hcy, the incidence of CVD, mortality from all causes and the onset of kidney failure dependent on dialysis did not differ between the two treatment arms [78]. A longitudinal ancillary study of the FAVORIT trial has recently indicated that the integration of high-dose B vitamins results in a modest cognitive benefit in patients with high base values. It should be noted that almost all subjects had no shortage of folate or B12, thus the potential cognitive benefits of folate and B12 supplementation in individuals with poor vitamin B status remain controversial [79].

Future perspectives and conclusion

At present, the results from available trials do not provide complete support for considering alterations in FA and vitamin B12 as reliable indices of CVD risk in CKD and ESRD population. Moreover, these factors do not represent a validated therapeutic target to cardiovascular risk reduction and CKD progression.

However, there is some evidence to indicate that the incidence of stroke and CKD progression might be controlled using more targeted FA therapy (baseline FA levels may have an impact on the efficacy of the FA intervention therapy), in particular among those with the MTHFR 677TT genotype and low to moderate folate levels and in countries without a grain fortification program [37,63]. However, in both general population and CKD patients, it remains a matter of debate if beneficial effects of FA therapy are due to its direct antioxidant effect or to a reduction in HHcy.

Discordant results in terms of CKD progression and cardiovascular risk, in the analyzed studies, result from differences in patient characteristics and FA treatment schemes among trials and may be influenced by the degree of cardiovascular and renal impairment.

In conclusion FA with or without vitamin B12 supplementation is an appropriate adjunctive therapy in patients with CKD and ESRD on dialysis treatment, in these cases FA may be supplemented pharmacologically after careful evaluation of folate status.

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