

Cobalamin deficiency, hyperhomocysteinemia, and dementia

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Introduction: Although consensus guidelines recommend checking serum B12 in patients with dementia, clinicians are often faced with various questions: (1) Which patients should be tested? (2) What test should be ordered? (3) How are inferences made from such testing? (4) In addition to serum B12, should other tests be ordered? (5) Is B12 deficiency compatible with dementia of the Alzheimer's type? (6) What is to be expected from treatment? (7) How is B12 deficiency treated?

Methods: On January 31st, 2009, a Medline search was performed revealing 1,627 citations related to cobalamin deficiency, hyperhomocysteinemia, and dementia. After limiting the search terms, all abstracts and/or articles and other references were categorized into six major groups (general, biochemistry, manifestations, associations and risks, evaluation, and treatment) and then reviewed in answering the above questions.

Results: The six major groups above are described in detail. Seventy-five key studies, series, and clinical trials were identified. Evidence-based suggestions for patient management were developed.

Discussion: Evidence is convincing that hyperhomocysteinemia, with or without hypovitaminosis B12, is a risk factor for dementia. In the absence of hyperhomocysteinemia, evidence is less convincing that hypovitaminosis B12 is a risk factor for dementia. B12 deficiency manifestations are variable and include abnormal psychiatric, neurological, gastrointestinal, and hematological findings. Radiological images of individuals with hyperhomocysteinemia frequently demonstrate leukoaraiosis. Assessing serum B12 and treatment of B12 deficiency is crucial for those cases in which pernicious anemia is suspected and may be useful for mild cognitive impairment and mild to moderate dementia. The serum B12 level is the standard initial test: 200 picograms per milliliter or less is low, and 201 to 350 picograms per milliliter is borderline low. Other tests may be indicated, including plasma homocysteine, serum methylmalonic acid, antiparietal cell and anti-intrinsic factor antibodies, and serum gastrin level. In B12 deficiency dementia with versus without pernicious anemia, there appear to be different manifestations, need for further workup, and responses to treatment. Dementia of the Alzheimer's type is a compatible diagnosis when B12 deficiency is found, unless it is caused by pernicious anemia. Patients with pernicious anemia generally respond favorably to supplemental B12 treatment, especially if pernicious anemia is diagnosed early in the course of the disease. Some patients without pernicious anemia, but with B12 deficiency and either mild cognitive impairment or mild to moderate dementia, might show some degree of cognitive improvement with supplemental B12 treatment. Evidence that supplemental B12 treatment is beneficial for patients without pernicious anemia, but with B12 deficiency and moderately-severe to severe dementia is scarce. Oral cyanocobalamin is generally favored over intramuscular cyanocobalamin.

Keywords: Alzheimer, dementia, cognitive impairment, cognitive dysfunction, cobalamin, cyanocobalamin, B12, homocysteine, hyperhomocysteinemia, homocystinuria

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Introduction

The notion of vitamin B12 deficiency (ie, B12 hypovitaminosis), its psychiatric and neurological manifestations, and its treatment is an age-old issue that has generated much controversy over many years. Although consensus guidelines¹⁻⁴ recommend checking serum B12 levels in patients with dementia, clinicians are often faced with questions regarding how to interpret and what to do with the results.⁵ In order to help in clarifying these uncertainties, six questions were posed and then answered: 1) In which patients should vitamin B12 be routinely assessed? 2) What test or tests should be ordered, and how are inferences made from such testing? 3) Does the finding of low serum B12 or elevated homocysteine (Hcy) require evaluation for other medical conditions? 4) When vitamin B12 deficiency is found in dementia, is dementia of the Alzheimer's type (DAT) a compatible diagnosis? 5) Based on the benefit-to-risk ratio in treatment of dementia, if vitamin B12 deficiency is determined, should supplemental B12 be initiated? 6) How is vitamin B12 deficiency treated?

By assimilating data from *in vitro*, *in vivo* animal, and *in vivo* human studies and epidemiological studies, this article clarifies these and other issues regarding hypovitaminosis B12, hyperhomocysteinemia (HHcy), and dementia.

Methods/results

The study design is a qualitative and quantitative review of the literature. The problem discussed is that in clinicians' geriatric practices, patients with dementia and low vitamin B12 were not showing significant improvement with supplemental B12 therapy. Our hypothesis is that patients with dementia and low vitamin B12 improve with supplemental B12 therapy. The null hypothesis is that patients with dementia and low vitamin B12 do not improve with supplemental B12 therapy.

On January 31st, 2009 a Medline search was performed using the search terms: (Alzheimer OR Alzheimer's OR dementia OR cognitive impairment OR cognitive dysfunction) AND (cobalamin OR cyanocobalamin OR B12 OR B-12 OR B 12 OR homocysteine OR hyperhomocysteinemia OR homocystinuria), which revealed 1,627 citations. "Title/Abstract" field limits decreased the search to 1,095 citations, which included 230 review articles. Using a Boolean operation, the review articles were removed, reducing the search to 865 citations. Subsequently, the search was limited to only citations with abstracts, so as to exclude publications such as 'Letters to the Editor' and case reports, which revealed 824 citations. Furthermore, in order to not miss any positive findings of individual case reports or case series, on September 6th, 2009

another Medline search was performed using the search terms: pernicious anemia AND dementia AND (case report OR case series) revealing 20 citations. Of the 844 articles, all abstracts were reviewed and, when useful, relevant articles were obtained and reviewed. Bibliographies from relevant articles were reviewed and, when applicable, review articles, 'Letters to the Editor,' and case reports were included in the overall review. Data from (1) other Medline searches, (2) Internet searches, (3) basic and clinical science textbooks, and (4) personal communications were added for clarification of technical issues. All abstracts, articles, and other references were categorized, allowing duplications, into six major categories: (1) general information, (2) biochemical evidence suggesting that hypovitaminosis B12 or HHcy are causal factors in dementia, (3) clinical and radiological manifestations, (4) associations between hypovitaminosis B12 or HHcy and cognitive impairment, (5) evaluation, and (6) treatment. Endnote version X.0.2 (Thomson Reuters, Philadelphia, PA) was used to maintain the reference library, which contained 839 citations. Evidenced-based medicine was used to develop suggestions for vitamin B12 workup and treatment in patients with suspected mild cognitive impairment (MCI) or dementia.

There are 511 articles and other references relevant to the six questions posed in the introduction and the six major categories listed above. Question 5 in the Introduction considers treatment benefits and risks. In terms of treatment benefits, the study objective was to determine whether or not vitamin B12 is beneficial for B12-deficient dementia. Letting the null hypothesis be, "Patients with dementia and low vitamin B12 do not improve with supplemental B12 therapy," not rejecting the null hypothesis when it is not true would be a type 2 error (false negative). In order to avoid a type 2 error, thus concluding B12 treatment is not beneficial, when in truth it is beneficial, all published studies and reports contained in Medline (Box 1), including case series, in which supplemental B12 was an exposure and cognitive change was an outcome are included in the discussion and tables (N = 38), regardless of the quality of the study or number of subjects. Also included are all published cohort and longitudinal studies in Medline, where exposure pertains to metabolic or serum B12 deficiency and outcomes pertain to change in cognitive function or development or prevention of dementia (N = 37). Also included are the majority of the retrospective and cross-sectional studies in Medline that examine similar outcomes and exposures. Articles pertaining to genetics, biochemistry, pathophysiology, clinical manifestations, and radiological manifestations illuminating our understanding on relationships between HHcy with and without cobalamin

Box I Studies and reports in which supplemental B12 is an exposure and cognitive change is an outcome

Abyad, 2002	Fox, 1975	McMahon, 2006
Aisen, 2008	Healton, 1991	Nilsson, 2001
Bolaman, 2003	Hvas 2004	Osmani, 2005
Bryan, 2002	Ikedo, 1992	Remington, 2009
Carmel, 1995	Kalita, 2008	Seal, 2002
Clarke, 2003	Kwok, 1998	Stott, 2005
Crystal, 1994	Kwok, 2008	Sun, 2007
Cunha, 1990	La Rue, 1997	Teunisse, 1996
Cunha, 1995	Lehmann, 2003	van Asselt, 2001
Eastley, 2000	Levine 2006	van Dyck, 2008
El Otmani, 2008	Lin, 2008	van Uffelen, 2008
Eussen, 2006	Lindenbaum, 1988	Wolters, 2005
Fourniere, 1997	Martin, 1992	

deficiency and dementia are included in the review, as are articles relevant to evaluation, prognosis, and treatment.

Discussion

General information

Vitamin B12 is composed of a central cobalt atom, attached to a dimethylbenzimidazole group, four nitrogen atoms, each pertaining to four pyrrole rings, and an R group (-CN, -OH, -CH₃, or adenosyl group), denoting the specific type of cobalamin.⁶⁻⁸ The definition of vitamin B12 deficiency is a quantitative lack of vitamin B12 in the diet, body fluids, or cells or a qualitative lack of intracellular B12 utilization.

B12 deficiency occurs in roughly 10% of general⁹⁻²³ and 17% of demented^{10,24-26} elderly populations. B12 deficiency in demented individuals ranges from one^{10,27,28} to five^{25,29} times that of controls. Serum B12 levels and cerebral spinal fluid (CSF) folate levels decrease with advancing age,^{9-12,30-34} whereas serum folate levels may either increase or decrease with age.³² Hcy is a nonessential thiol amino acid.^{35,36} HHcy is defined as an abnormally high level of total Hcy in the plasma. HHcy occurs in 6% to 81% of individuals, depending on the population studied.³⁷⁻⁴⁴ Causes of HHcy and B12 deficiency are listed in Table 1.^{7,18,19,39,45-62} Further details regarding differential diagnoses are available on the Internet.⁶²

Biochemical evidence suggesting that hypovitaminosis B12 or hyperhomocysteinemia are causal factors in dementia

The means by which HHcy is involved in dementia may possibly be explained by aging and reduced vitamin B12

Table I Causes of hyperhomocysteinemia and B12 deficiency

Causes of hyperhomocysteinemia

- Genetic deficiencies and polymorphisms^{52,58}
 - methylenetetrahydrofolate reductase
 - methionine synthetase
 - cystathionine beta-synthetase
- Drugs
 - levodopa (L-dopa) – due to its metabolism^{46,51,54}
 - nitrous oxide – due to methionine synthetase inhibition^{48,49,53}
 - others
- Nutritional deficiencies
 - vitamin B6
 - folate
 - vitamin B12 (caused by pernicious anemia, celiac disease, and others)
- Other factors^{7,18,19,39,52,57,59-61}
 - advanced age
 - male gender
 - renal insufficiency

Causes of B12 deficiency^{45,47,50,55,56,62}

- Genetic deficiencies of intrinsic factor – due to inability to absorb B12 at the distal ileum
- Genetic deficiencies or polymorphisms of transcobalamin II – due to inability or impediment of B12 cellular uptake
- Diseases affecting salivary glands – due to R-protein hyposecretion
- Gastric disease, resection, or bypass surgery – due to lack of hydrochloric acid and pepsin, which are required to separate animal protein from dietary B12, causing food-cobalamin malabsorption (eg, *Helicobacter pylori* infection)
- Diseases affecting gastric parietal cells – due to hyposecretion and inhibition of intrinsic factor (eg, pernicious anemia)
- Diseases affecting the pancreas and upper small intestine – due to lack of pancreatic enzymes and insufficient elevation of pH, which are required to separate R protein from B12 (eg, pancreatic insufficiency and ileal resection)
- Ileal disease or resection – due to specific inability to absorb the intrinsic factor-B12 complex (eg, celiac disease)
- Various malabsorption syndromes
- Medications
 - histamine type 2 receptor blockers
 - protein pump inhibitors
- Dietary deficiencies (eg, vegetarian diet)
- Advancing age

or folate supply versus demand ratio,^{63,64} with functional, structural, genetic, and nutritional determinants. Possible functional determinants include Hcy agonism of N-methyl-D-aspartic acid (NMDA) receptors, which causes excessive intracellular calcium influx and neuronal death^{65,66} and HHcy creating a state of hypomethylation in the pathogenesis of Alzheimer's disease (AD),^{21,58,67} causing deoxyribonucleic acid (DNA) damage and apoptosis.⁶⁸⁻⁷¹ HHcy inhibits adult mammal hippocampal neurogenesis.⁷² Hcy may compete with gamma-amino butyric acid (GABA) at the GABA receptor and may affect its inhibitory function.^{73,74}

Potential structural determinants include HHcy causing blood–brain barrier (BBB) dysfunction^{75–78} and endothelial cell toxicity,^{47,64} whereby Hcy changes endothelial cell surface properties from anticoagulant to procoagulant.^{47,52,73,79} Genetic determinants include various polymorphisms and homozygous monogenic deficiencies, involving methylenetetrahydrofolate reductase (MTHFR), transcobalamin (TC) II, methionine synthetase (MS), and cystathione beta-synthetase (CBS). These may contribute to hypovitaminosis B12, HHcy, and dementia.^{7,47,52,64,73,80–82} Meta-analyses^{83–89} of the MTHFR polymorphism show that those with 677 TT alleles compared to 677 CC alleles have elevated Hcy and increased risk for myocardial infarction (MI), transient ischemic attack, and stroke. Hyperhomocysteinemic individuals with certain butyrylcholinesterase-K (BuChE-K) alleles cognitively decline more rapidly than those with wild-type BuChE alleles.⁹⁰ Nutritional determinants include decreased vitamin B12 ingestion^{9,45,47} and food-cobalamin malabsorption.^{9,15,19,45,49,91–93}

In vitro, *in vivo* animal, and *in vivo* human studies suggest AD pathophysiology conceivably involves a hypomethylation state,^{67,94–97} reactive oxygen species (ROS) generation,^{27,98–101} immune activation,^{102–110} and anomalous protein development.¹¹¹ Abnormal Hcy metabolism is involved in each of these four processes. Hcy is metabolized by the methionine cycle and transsulfuration pathway (Figure 1),¹¹² where it has one of three possible fates: methylation to methionine (Met), transsulfuration to cystathionine, or adenosylation to S-adenosylhomocysteine (SAH).^{113,114} With normal Hcy levels, the first two reactions are maintained, the first occurring in the brain and body and the second predominately occurring in the body, promoting homeostatic methylation reactions and maintaining healthy cells. With Hcy elevation the third reaction ensues, promoting a hypomethylation state leading to disease.¹¹⁵

On the one hand, without oxidative chemical reactions, as the basis for cellular respiration, we would not have life, at least as we know it. On the other hand, without these reactions, we would not have ROS.¹¹⁶ There are thousands of publications related to ROS and aging, the greatest known risk factor for sporadic AD.^{114,116,117} Oxidative metabolism generates a very small fraction of ROS,¹¹⁷ which can be beneficial or detrimental to the central nervous system. Oxidative stress occurs when ROS generation exceeds ROS defense,¹¹⁸ leading to potential molecular and cellular damage.¹¹⁷ Aberrant mitochondrial enzymes may facilitate this process, thereby contributing to the pathophysiology of AD.¹¹⁶

Hcy is rapidly auto-oxidized to homocysteine thiolactone, homocystine, and mixed disulfides,^{7,119} producing ROS,^{18,21,47,52,68,120} including singlet oxygen, superoxide anions, hydroxyl radicals, and hydrogen peroxide.^{21,42,52,68,117,118} Hcy elevation is associated with microglia activation and proliferation⁷¹ and immune activation and deposition,¹⁰⁸ which is associated with choroid plexus dysfunction,^{121–123} possibly impeding vitamin B12^{123–125} and folate^{122,125} influx to, and amyloid beta (A β) peptide^{123,126,127} clearance from, brain tissue in AD. In certain systems, Hcy elevation leads to amyloid precursor¹²⁸ and tau^{128–132} protein hyperphosphorylation, and Hcy oxidation produces products that crosslink with A β and tau proteins, causing their precipitation.^{119,128,133} Hcy elevation is associated with increased A β peptide, in both brain¹³⁴ and plasma.^{51,135,136}

A deleterious cycle may occur between ROS generation and immune activation, where the former may cause the latter^{137–139} and vice versa.^{63,64,140–144}

Hcy may promote a means for such a cycle,^{64,106,120} because ROS generation is associated with Hcy elevation,^{120,142,145–147} which is associated with immune activation,^{64,71,79,148–150} and immune activation is associated with Hcy elevation,^{64,142,151,152} which is associated with ROS generation (Figure 2).^{47,64,71,153–155} Additional evidence verifies ROS,^{145,151,156} hypomethylation,^{21,58,67,94,95,128,136,157} immunological components,^{64,79,106,109,110,142} and A β and tau proteins^{58,65,66,120,136,158} interact with one another, producing a neurodegeneration cascade in AD. ROS generation precedes both A β peptide deposition^{159–162} and tau-associated neurodegeneration.¹⁶³ ROS generation deregulates tau protein phosphorylation,^{164,165} and is associated with decreased S-adenosylmethionine (SAM),¹²⁰ increased SAH, a decreased SAM/SAH ratio,¹¹⁵ and hyperconsumption and depletion of antioxidants^{98,151,156} and tetrahydrofolate (THF).^{64,120,151,156,166}

With absolutely or relatively low folate or vitamin B12, MS-mediated Hcy clearance is impeded,^{52,120} resulting in a hypomethylation state. Also, Hcy elevation may lead to hyperconsumption and depletion of vitamin B12 in various cases of AD and vascular dementia (VaD).¹⁴⁶ Hcy potentiates A β peptide-induced ROS generation and apoptosis.^{69,154,155,167} A β and tau proteins are concentrated sources for further ROS generation, Hcy elevation, and immune activation, thereby perpetuating the deleterious cycle.^{120,143,168–170} Also, *in vivo* human studies show that free cobalt is elevated in individuals with AD compared to controls.⁸ *In vitro* studies show free cobalt generates oxidative stress, as measured by reduced

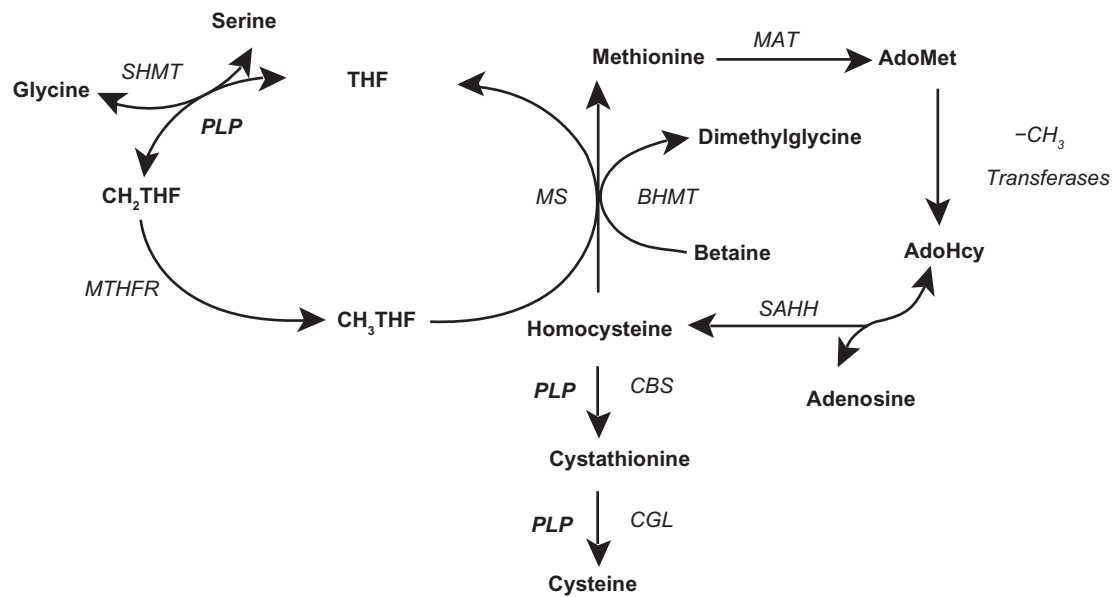


Figure 1 Folate cycle, methionine cycle, and transsulfuration pathway. Copyright © 2005. Adapted with permission from Davis SR, Quinlivan EP, Shelnutt KP, et al. Homocysteine synthesis is elevated but total remethylation is unchanged by the methylenetetrahydrofolate 677C->T polymorphism and by dietary folate restriction in young women. *J Nutr.* 2005;135(5):1045–1050.

Notes: This schematic is of the folate cycle (left), methionine cycle (right), and transsulfuration pathway (bottom), with homocysteine being the common substance to all three. Folic acid (synthetic) is converted by DHFR to dihydrofolic acid (dietary), which is converted by DHFR to THF, which enters the folate cycle: THF → N⁵,N¹⁰-methylene THF → N⁵-methyl THF → THF. In this last step, vitamin B12 is required as a cofactor for MS. With low or absent vitamin B12, this last step is hindered leading to the methylfolate trap with elevated CH₃-THF. Homocysteine metabolism: homocysteine is produced in the methionine cycle by the deadenosylation/hydration of AdoHcy, and is either remethylated to methionine, by the methionine cycle or catabolized to cysteine, by the transsulfuration pathway. Note the AdoHcy deadenosylation/hydration to Hcy is a reversible reaction favoring homocysteine adenosylation/hydration to AdoHcy. Methyl groups produced by AdoMet demethylation to AdoHcy are used for nucleic acid, protein, lipid, and neurotransmitter biosynthesis. Cysteine is a nonessential amino acid used in the biosynthesis of proteins, glutathione, coenzyme A, taurine, and inorganic sulfur. Glutathione is an antioxidant that protects cells from ROS.

Abbreviations: AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β-synthase; CGL, cystathionine gamma-lyase; CH₂-THF, methylenetetrahydrofolate; -CH₃, methyl group; CH₃-THF methyl tetrahydrofolate; DHFR, dihydrofolate reductase; MAT, methionine adenosyltransferases; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; PLP, pyridoxal phosphate (the active form of vitamin B6, pyridoxine); ROS, reactive oxygen species; SAHH, S-adenosylhomocysteine hydrolase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

glutathione, increases Aβ peptide secretion, and produces neuroblastoma cytotoxicity.^{8,120}

Within the central nervous system, a balance may occur between endogenous neurotoxic agents on one hand and endogenous neurotrophic agents on the other hand.¹⁷¹ Examples of potential neurotoxic agents include tumor necrosis factor-alpha (TNF-α), nerve growth factor

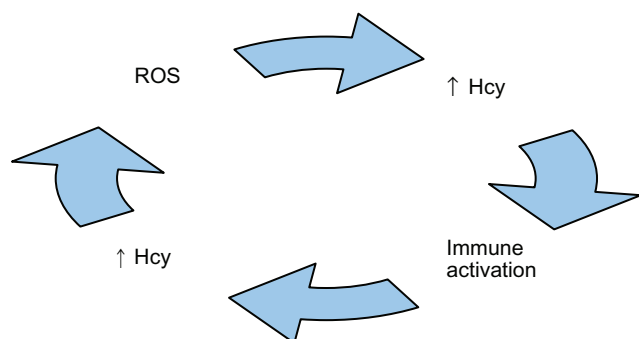


Figure 2 Illustration of a biologically plausible deleterious cycle of reactive oxygen species (ROS), homocysteine (Hcy), and immune activation that possibly may be involved in the pathogenesis of Alzheimer's disease.

(NGF), and the soluble CD40-soluble CD40 ligand dyad (sCD40-sCD40). Examples of potential neurotrophic agents include interleukin-6 (IL-6), epidermal growth factor (EGF), and transforming growth factor-beta1 (TGF-β1). In animals, if the balance is tilted in favor of TNF-α, NGF, and sCD40-sCD40 (eg, by the administration of exogenous TNF-α),¹⁷² as opposed to IL-6, NGF, and TGF-β1, then the morphological changes of subacute combined degeneration (SCD) are observed: white matter interstitial edema, intra-myelinic edema, spongy vacuolation, and astrogliosis.^{172–175} Vitamin B12-depleted animals exhibit increased levels of TNF-α, NGF, and sCD40-sCD40^{172,176} and decreased levels of IL-6 and EGF,¹⁷⁶ thereby tilting the balance and developing the myelopathic changes of SCD. Not only does treatment with vitamin B12 reduce or reverse these changes,^{172,175} but treatment with anti-TNF-α antibodies, IL-6, EGF, and TGF-β1 does so as well.^{172,176} Interestingly, a similar observation has been observed in humans. Serum TNF-α is higher and serum EGF is lower in subjects with severe B12 deficiency compared to controls, where a

direct correlation is found between plasma Hcy and serum TNF- α .¹⁷³ The association is translatable into the CSF, where CSF B12 and EGF are lower and CSF Hcy and TNF- α are higher in subjects with SCD compared to non-B12 deficient controls.¹⁷⁷ B12-repletion lowers serum TNF- α and raises serum EGF, thereby normalizing the imbalance, which occurs concomitantly with clinical and hematological disease remission.¹⁷³ Hence, in addition to well-known enzymatic roles for vitamin B12, it is thought to also have nonenzymatic roles, where it is associated with downgrading synthesis and release of TNF- α and upgrading synthesis and release of EGF.^{173,177,178}

Clinical and radiological manifestations

Clinical manifestations of low vitamin B12 include abnormal psychiatric, neurological, and gastrointestinal findings. Psychiatric manifestations consist of psychoses, including paranoia, delusions, and hallucinations,^{23,58,179–184} cognitive dysfunction, including memory impairment, delirium, and dementia,^{7,21,23,49,58,93,180,181,185–188} and affective syndromes, including mania and depression,^{7,21,23,58,93,180,181,186,189–191} which also occurs with elevated Hcy^{192–194} and low SAM.^{67,192} Associations exist between HHcy and cognitive dysfunction in bipolar disorder^{195–197} and perhaps schizophrenia.¹⁹⁸ Neurological manifestations include myelopathy and peripheral, autonomic, and optic neuropathies.¹⁹⁹ Paresthesia is caused by a sensory lesion anywhere between the peripheral nerve and brain and is often the initial symptom.^{23,58,92,180,181,188} SCD refers to myelopathy affecting posterior and lateral columns, characterized by a pernicious sequence of vacuolar demyelination, axonal degeneration, and neuronal death.^{18,58,200–202} Posterior column myelopathy affects afferent pathways, causing the most common neurological signs: ataxia, diminished proprioception and vibratory senses, and presence of Romberg's sign.^{18,49,58,180,185,188,200,201,203} Lateral column myelopathy affects efferent pathways, causing the second most common neurological signs: extremity muscular weakness, spasticity, hyperactive reflexes, and Babinski's sign.^{18,49,58,180,181,200,201} Peripheral and autonomic neuropathies cause hypoactive reflexes, sensory loss, orthostatic hypotension, fecal and urinary incontinence, and impotence.^{18,23,49,58,92,180,181,185,188,200,203} Although optic neuropathy is uncommon,^{23,58,180,203} visual impairment may occasionally be the earliest or sole manifestation of the disease.²⁰³ Gastrointestinal manifestations include epithelial atrophy of the tongue, referred to as atrophic glossitis, which causes the tongue to be sore and beefy red,^{18,23} and epithelial atrophy of the stomach.¹⁵

Computerized axial tomographic (CAT) and magnetic resonance imaging (MRI) scans of nondemented elderly brains may show age-related cerebral atrophy and various grades of periventricular white matter disease consistent with chronic microvascular ischemia.^{204–208} Although linear and volume measurement methods, evaluating ventricle-to-brain ratios and medial temporal lobe atrophy, reveal significant differences in group means, between those with AD and controls,^{205,207,209–217} such strategies are not recommended for the purpose of diagnosing AD.^{3,218} Assuming normal distributions of both nondemented and demented groups, a certain degree of overlap may exist,^{205,207,216} unless specified by scan angle-adjusted temporal lobe neuroimaging.^{207,214} Even so, such imaging may not distinguish non-Alzheimer's dementia from controls.²¹⁷ Although CAT scans of nondemented elderly commonly show age-related cerebral atrophy, those of demented elderly often show cerebral atrophy more than expected for age,²¹⁰ and are read as variably judged atrophy and differently interpreted white matter changes.^{218,219} Whether or not such findings relate to dementia with low vitamin B12 and/or elevated Hcy is often unclear.

Accordingly, a literature review finds radiological manifestations of low vitamin B12 and/or elevated Hcy to include leukoaraiosis, brain atrophy, and silent brain infarcts.^{199,220–237} Leukoaraiosis is a radiological term that refers to brain white matter hypodensity on CAT scans or hyperintensity on T₂-weighted magnetic resonance imaging (MRI).²³⁸ It is associated with aging,^{221,230,239–244} chronic microvascular hypoperfusion,²⁴⁵ BBB dysfunction,²⁴⁶ hypertension,^{221,230,240,241,242,244} stroke,^{230,239,240–242,244,247} and death.²³⁹ Leukoaraiosis is described pathologically as periventricular leukoencephalopathy or subcortical arteriosclerotic encephalopathy; it occurs in brains of individuals with AD²⁴³ and dementia of the Binswanger type (DBT),²⁴⁸ which is a relatively rarer type of dementia and is associated with other findings. HHcy increases the risk for leukoaraiosis.^{220,221,223–226,228,230,232,236} Independently from plasma Hcy levels, one cross-sectional study²⁴⁹ found an inverse association between the concentration of normal range serum B12 levels and the degree of leukoaraiosis; nonetheless, in the absence of HHcy, two studies^{227,231} found low serum B12 does not increase the risk for leukoaraiosis. Parenthetically, one prospective study²⁵⁰ found an inverse association between serum folate levels and the degree of leukoaraiosis. Although leukoaraiosis is more prevalent in demented than nondemented individuals^{225,247} and it increases the risk for developing dementia,^{247,248,251} results are mixed in terms of whether or not the link between HHcy and cognitive dysfunction is specifically mediated by

leukoaraiosis.^{228,232,240,243,252–255} In both cross-sectional and prospective evaluations, hypovitaminosis B12 and HHcy are associated with brain atrophy.^{199,222,229,232,234,237} HHcy is associated with silent brain infarcts.²³²

VaD,⁶¹ DBT,²⁵⁶ and AD,^{61,222,243,257–259} represent various spectra of vascular pathology. To illustrate, cerebral amyloid angiopathy occurs in many cases of DBT²⁴⁸ and in most cases of AD,^{46,260,261} where increased vessel atrophy,²⁶² decreased microvascular density,²⁶² reduced temporal lobe blood flow,^{263,264} and spontaneous cerebral emboli^{265,266} are other significant findings. Hcy permeates through the BBB,^{267,268} causing BBB dysfunction,^{74,76–78} which is observed in AD,^{247,269} DBT,^{220,223,256,270} and VaD,⁷⁵ allowing easy influx for a wide variety of proteins to cerebral interstitial fluid^{271,272} and vice versa.²⁷³ With BBB dysfunction, brain parenchyma likely becomes less protected from toxic effects of systemic HHcy,²²⁹ which increases risk for acute macrovascular disease, or strokes,^{7,21,70,84,85,87,151,223,274–279} causing loss of volume, and chronic microvascular disease, or ischemia,^{135,220,280} causing loss of cortical-to-subcortical connections,²⁴⁸ occasionally with findings as those in DBT.^{220,223,281,282}

Associations between hypovitaminosis B12 or HHcy and cognitive impairment

HHcy, with hypovitaminosis B12, are common findings in the evaluation of MCI,^{59,283–288} AD,^{43,60,61,82,111,222,276,284,289–291} VaD,^{43,61,145,276,284,292} and other dementia subtypes.^{90,282,293,294} This raises the question of whether or not these findings are the cause of, result of, or unrelated to the disease process. Although multiple retrospective and cross-sectional studies find associations between hypovitaminosis B12 and HHcy and cognitive impairment with or without dementia,^{28,39,43,59–61,82,90,111,145,186,196,222,276,283–306} cohort studies are required to provide evidence that hypovitaminosis B12 or HHcy are risk factors for cognitive dysfunction. Eighteen^{106,136,252,258,301,307–320} of 22 cohort studies demonstrate HHcy either increases the risk for cognitive impairment or development of dementia. Depending upon whether plasma Hcy levels increase or decrease over time, the degree of change either increases the risk for developing dementia³¹³ or decreases the risk for poorer memory performance,³¹⁶ respectively. The greater the baseline plasma Hcy level, the faster the rate of cognitive decline.³²⁰ However, one study found that the elevated plasma Hcy risk for developing dementia diminished when controlling for low serum folic acid.³⁰¹ Four^{321–324} of 22 cohort studies conclude Hcy is not a factor in the development of MCI or dementia, although outcome assessment may have been a methodological weakness in

one of these.³²⁴ One cohort study³²⁵ concludes HHcy is a consequence of the development of cognitive impairment. Most,^{222,276,316,326–328} but not all,³²⁹ studies find HHcy is associated with intensity, rather than duration, of illness in AD. Thus, evidence that HHcy increases the risk for cognitive impairment and dementia usually is consistent and reproducible,²³³ with HHcy predictably increasing the risk that subjects with MCI will progress to dementia.²³³ With respect to the involvement of low vitamin B12, seven cohort studies^{308,312,313,330–333} show low vitamin B12 either increases the risk for cognitive impairment or development of dementia, while eight^{13,325,334–339} show no increased risk. Paradoxical findings in these studies include modest increases in serum B12 levels over time may increase the risk for dementia³¹³ and individuals with normal serum B12 levels having a higher incidence of AD compared to those with low serum B12 levels.¹³ Thus, evidence that low vitamin B12 increases the risk for cognitive impairment or dementia is inconclusive. Then again, the increased risk may occur in the opposite direction. AD may increase the risk for B12 deficiency.³⁴⁰

Evaluation

In which patients should vitamin B12 be routinely assessed?

Guidelines for the workup of dementia are listed in Figure 3.^{4,341} Although practices may range from checking serum B12 in all elderly to only particular patients with dementia, an evidence-based approach warrants checking serum B12 in all patients with MCI and mild to moderate dementia of two years or less duration. It is especially useful to assess serum B12 levels in all patients with MCI, because many will ultimately advance to dementia, where there may be a window of opportunity when vitamin B12 treatment of B12 deficiency-related cognitive dysfunction is potentially beneficial. Additionally, serum B12 should be checked in all patients with (1) history of gastric bypass surgery, partial or total gastrectomy, terminal ileum disease or resection, or pancreatic insufficiency,^{14,20,33,200} (2) chronic use of levodopa, histamine type-2 (H2) receptor blockers, or protein pump inhibitors (PPIs),^{22,33,51,54,342–345} or (3) findings suggestive of (a) behavioral and psychological symptoms of dementia (BPSD), (b) SCD, including paresthesias, ataxia, or loss of position or vibratory senses, or (c) pernicious anemia (PA), including low hemoglobin (Hgb), elevated mean corpuscular volume (MCV), or corpuscular changes on peripheral smear.⁶² If such findings are absent, and patients have moderately severe to severe dementia of longer than two years duration, then universal recommendations^{1–4} of

Guidelines for the workup of dementia, adapted from the American Academy of Neurology Guidelines on Dementia, 2004.

- (1) The DSM-III-R definition of dementia is reliable, and its use is appropriate.
- (2) The DSM-IV or the NINCDS-ADRDA diagnostic criteria for DAT and CJD have sufficient reliability and validity, and their use is appropriate.
- (3) VaD, DLB, and FTD should be excluded, but the current diagnostic criteria for those diseases have imperfect reliability and validity.
- (4) Screening for depression is indicated.
- (5) Structural neuroimaging, with either a noncontrast CAT scan or MRI scan of the brain, in the initial evaluation of patients with dementia, is appropriate in order to detect lesions that may result in cognitive impairment.
- (6) Evidence supports the following tests in the routine evaluation of the demented patient:
 - (a) Complete blood count
 - (b) Liver panel (or liver function test)
 - (c) Renal panel (or serum electrolytes, glucose, blood urea nitrogen, and creatinine)
 - (d) Serum B12 Level
 - (e) Thyroid-stimulating hormone (or thyroid function test)
 - (f) Urinalysis
- (7) Evidence indicates the following tests should not be included in the routine evaluation of the demented patient:
 - (a) Screening for syphilis (unless patient has risk factors such as living in a high-incidence region or engaging in behaviors at high risk for syphilis)
 - (b) Linear or volumetric MRI or CAT measurement strategies
 - (c) SPECT
 - (d) Genetic testing for DLB or CJD
 - (e) APOE genotyping for AD
 - (f) EEG
 - (g) Lumbar puncture (unless presence of metastatic cancer, suspicion of CNS infection, reactive serum syphilis serology, hydrocephalus, age under 55, rapidly progressive or unusual dementia, immunosuppression, or suspicion of CNS vasculitis)
- (8) At this time, there is not enough evidence to support or refute the use of the following tests:
 - (a) PET
 - (b) Genetic markers for AD not listed above
 - (c) CSF or other biomarkers for AD
 - (d) Tau mutations in patients with FTD
 - (e) AD gene mutations in patients with FTD

Figure 3 Guidelines for the workup of dementia.

Note: Excellent guidelines for the diagnosis of dementia also are available at <http://www.cmaj.ca/cgi/content/full/178/7/825>.

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; CAT, computerized axial tomography; CJD, Creutzfeldt-Jakob Disease; CNS, central nervous system; DAT, Dementia of the Alzheimer Type; DLB, Dementia with Lewy Bodies; DSM-III-R, *Diagnostic and Statistical Manual of Mental Disorders-III-Revised*; DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders-IV*; EEG, electroencephalogram; FTD, frontotemporal dementia; MRI, magnetic resonance imaging; NINCDS-ADRDA, National Institute of Neurologic, Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association; PET, positron emission tomography; SPECT, single photon emission computerized tomography; VaD, vascular dementia.

assessing for and, when found, treating B12 deficiency may not be supported by reliable evidence.^{13,16,24,26,92,187,326,346–364} H2 receptor blockers and PPIs impair cobalamin absorption;^{18,22,33,365,366} their use is associated with supplemental B12 initiation.³⁶⁷ Individuals who have had gastric surgery have a high prevalence of B12 deficiency,²⁰ and those who have had gastrectomies, who are B12-deficient, have a high prevalence of cognitive dysfunction and electroencephalographic (EEG) abnormalities.³³² Therefore, patients prescribed H2 receptor blockers or PPIs, and those who have had gastrectomies or gastric bypass surgery, require monitoring for hypovitaminosis B12.³⁶⁸

What test or tests should be ordered, and how are inferences made from such testing?

Common tests include the deoxyuridine suppression test (dUST), serum B12, serum TC II, plasma Hcy, serum or urinary methylmalonic acid (MMA), and the post-methionine load test. For convenience, conversion of vitamin B12 units (nanograms per liter to picograms per milliliter (pg/mL) and pg/mL to picomoles per liter) are available at <http://www.cdc.gov/ncbddd/b12/index.html>.

The dUST is probably the most sensitive and specific test for assessing functional folate or B12 deficiency.^{92,369} Although the test is not fully understood and probably more complex than a simple explanation,³⁶⁹ if incubated bone marrow cells or peripheral blood lymphocytes have sufficient folate and cobalamin, nonradioactive deoxyuridine is believed to suppress the thymidylate synthetase conversion of radioactive thymidine into thymidylate, which is a normal response, but if cells have insufficient folate or cobalamin, nonradioactive deoxyuridine does not suppress the thymidylate synthetase conversion of radioactive thymidine into thymidylate, which is an abnormal response.

The most common test is the serum B12 level,¹⁵ but often it is difficult to determine which serum B12 levels represent deficiency states and which do not. When using a specific serum B12 cutoff point, for example 200 pg/mL or less,^{22,181,200,368} in determining which individuals do and do not have B12 deficiency, problems encountered include false positives and false negatives.^{7,15,19,30,33,57,188,370–376} One of the reasons for this is because most serum B12 assays measure total B12, including free B12 and that bound to B12 binding proteins: TC I, also called haptocorrin, TC II, simply referred to as transcobalamin, and TC III, which is produced by neutrophils.^{7,371,374} TC I and III are R-proteins (R for rapid movement on electrophoresis).^{7,23,200,377} TC I is a storage protein and does not participate in cellular uptake.^{200,377}

TC II participates in all cellular uptake.^{7,58,200,371,372,377} TC III participates in hepatocyte uptake only.²⁰⁰ TC I and II comprise about 80% and 20% of total B12, respectively. Falsely low serum B12 occurs in congenital TC I deficiency, where TC I is low or absent, but TC II, intracellular B12, and hematopoiesis are normal.^{7,56,200,377} Other causes of falsely low serum B12 include folate deficiency, multiple myeloma, oral contraceptive use, and pregnancy.^{7,56} The mechanism by which low serum folate causes falsely low serum B12 is poorly understood.^{7,368} Alternatively, low serum cobalamin causes methyltetrahydrofolate (CH₃-THF) trapping, elevating serum CH₃-THF, which causes falsely high serum folic acid (Figure 1).^{58,200,372,378} Falsely normal serum B12 occurs in congenital TC II deficiency, where TC I is normal, but TC II is low or absent, and intracellular B12 is insufficient, causing severe megaloblastic, macrocytic anemia.^{7,56,200,377,379} Other causes of falsely normal serum B12 include liver disease, myeloproliferative disorders, and intestinal bacterial overgrowth.^{7,56} Since serum TC II levels decrease with advancing age, a given serum B12 level in an elder may represent a deficiency, compared with the same level in a younger adult.^{30,380} Directly measuring serum TC II may be helpful in these cases and others.^{17,38,308,335,351,372,374,380–383}

Studies show 10%^{56,203} to 50%^{19,368} of truly vitamin B12 deficient individuals have serum B12 between 200 and 350 pg/mL, presenting clinicians with a diagnostic challenge. Based upon known biochemistry, specific tests can be ordered to help with this challenge. Cytoplasmic methylcobalamin is needed for MS-catalyzed Hcy methylation to Met, and mitochondrial adenosylcobalamin is needed for L-methylmalonyl-CoA mutase-catalyzed L-methylmalonyl-CoA conversion to succinyl-CoA.^{7,18,19,20,52,57,58,203} With low cellular B12, these reactions are impeded, elevating Hcy in the former and L-methylmalonyl-CoA, D-methylmalonyl-CoA, and MMA in the latter.^{18,20,285} Thus, HHcy and hypermethylmalonic acidemia (HMMA) are surrogate markers for low vitamin B12 cellular levels (ie, metabolic B12 deficiency).^{7,11,15,19–22,30,33,44,59,60,188,203,285,294,351,353,368,370,372,373,376,380,384,385} HHcy occurs with deficiency of pyridoxine, folate, or vitamin B12;^{19,306,355,370,386} usually, only deficiency of vitamin B12 causes HMMA.^{19,20,370} Hcy may be the more sensitive and MMA the more specific surrogate marker.^{17,20,38,40,151,156,285,387–389} Either surrogate marker is more sensitive than serum B12 in evaluating PA,^{7,19,373,390} and many individuals with HHcy have normal serum folate and B12 levels.^{38,39,42} When used alone, B12,^{371,375} TC II,³⁷¹ Hcy,³⁸ or MMA³⁸⁹ may be insufficient as screening tests, but in combination, normal plasma Hcy and serum MMA rule out B12 deficiency in virtually all

cases.^{19,390} Although the post-methionine load test is twice as sensitive as basal Hcy in detecting HHcy in individuals with AD,³⁹¹ it is not recommended as an initial test.¹⁷

Most laboratory assays measure total Hcy, including reduced and oxidized forms: Hcy representing the former and homocystine and mixed disulfides (protein-bound Hcy and cysteine-Hcy) the latter.⁵² Although the upper reference limit for plasma Hcy varies according to different conditions,^{17,33} plasma Hcy greater than 14 $\mu\text{mol/L}$ in patients with vitamin B12 levels between 201 and 350 pg/mL suggests cellular B12 deficiency.^{23,41,200,222,252,258,357,387,392} Serum B12 greater than 350 pg/mL rules out B12 deficiency in almost all individuals.^{56,200,340} Since tissues store vitamin B12 for up to five years^{18,368,393} and plasma Hcy increases only minimally after protein-rich meals,¹⁷ serum B12 and plasma Hcy may be obtained fasting or nonfasting. Immediate centrifugation, or keeping samples refrigerated or ice cooled until centrifugation, prevents spuriously elevated Hcy results.^{17,394,395} Suspected B12 deficiency can be confirmed when individuals having characteristic findings, including anemia, macrocytosis, corpuscular changes on peripheral smear, or signs and symptoms of SCD or peripheral neuropathy, improve with vitamin B12 treatment or when elevated Hcy or MMA are lowered with vitamin B12 treatment.^{19,20,92,135,188,347,362,370,392,396,397} Although not intended to replace clinical judgment, suggestions for vitamin B12 workup and treatment in patients with suspected MCI or dementia are presented (Figure 4).

Does the finding of low serum B12 or elevated Hcy require evaluation for other medical conditions?

Some authors recommend establishing the etiology of B12 deficiency as part of the diagnostic approach.⁵ It is beyond the scope of this paper to cover all diagnostic considerations in Table 1, but levodopa treatment, folate deficiency, and PA are noteworthy.

In Parkinson's disease (PD) it is the treatment rather than the disease that causes HHcy.^{37,54,294} In such cases, HHcy may^{293,294} or may not³⁹⁸ be associated with cognitive impairment. Recall from Figure 1, Met is converted to SAM, which is converted to SAH, which is converted to Hcy. SAM demethylation to SAH serves as a methyl group donor for the biosynthesis of nucleic acids, proteins, phospholipids, and catecholamines and the metabolism of drugs and toxins. Catechol-O-methyl transferase (COMT) catalyzes levodopa methylation to 3-O-methyldopa.³⁴⁴ Increased methyl group demand, required for the methylation of levodopa to 3-O-methyldopa, favors the conversion of Met to SAM, demethylation to SAH,

conversion to Hcy, and development of HHcy.^{51,54,342,344} In a cross-sectional study,³⁴² individuals on levodopa alone, compared to those on combined levodopa and COMT inhibitor therapy, had higher plasma Hcy levels. However, only one³⁴⁴ of three^{54,343,344} prospective studies showed addition of a COMT inhibitor to those on levodopa prevents dopamine-associated plasma Hcy elevation or serum B12 reduction.

Since the relationship between folate and vitamin B12 is biochemically and, in deficiency states, pathologically united,²⁸⁵ there are caveats to keep in mind when working up and treating folate and B12 deficiencies. When folate and B12 deficiencies occur together, monotherapy with either folate or vitamin B12 can worsen the manifestations of the other vitamin deficiency.^{7,23,180,285,303,355,371,399,400} Thus, it is beneficial to assess serum levels of both vitamins and treat whichever deficiency occurs. Although PA and SCD share the common etiology, type A (autoimmune) chronic atrophic gastritis, with vitamin B12 malabsorption, they have distinct pathophysiologies.^{18,201} In the methionine cycle, folate- and vitamin B12-dependent MS catalyzes Hcy methylation to Met, as CH₃-THF, serving as the methyl group donor, is demethylated to THF.^{47,52,58,151,200} Impairment of this reaction causes defective DNA synthesis, leading to megaloblastic, macrocytic anemia (ie, PA).^{21,58} Supplemental folic acid can override the impairment, restoring DNA synthesis and normalizing erythropoiesis.^{58,401} Furthermore, in the methionine cycle, Met is adenosylated to SAM, which is demethylated to SAH, which is deadenosylated to Hcy.⁵⁸ Impairment of SAM demethylation to SAH impedes methylation reactions, leading to vacuolar demyelination, axonal degeneration, and neuronal death (ie, SCD).^{21,58} Supplemental folic acid cannot override the methylation impairment, resulting in progressive neuropathology and neuronal death.⁵⁸ Treating a combined folate and B12 deficiency with folic acid alone may correct hematological abnormalities, but not neurological abnormalities, and can aggressively worsen B12-deficient neurological sequelae.^{7,18,19,23,200,203,355} Thus, B12 deficiency should be ruled out before correcting folate deficiency.

When folate and vitamin B12 are in balance, increased serum folate levels are associated with decreased plasma Hcy and serum MMA levels, but when vitamin B12 is underrepresented, increased serum folate levels are associated with increased plasma Hcy and serum MMA levels.²⁸⁵ Also, higher folate states require relatively higher vitamin B12 levels than normal folate states to protect against metabolic B12 deficiency.²⁸⁵ Therefore, the borderline (201–350 pg/mL) serum B12 range may be higher in the presence of high folate states.

Suggestions for vitamin B12 workup and treatment in patients with suspected neuropathology

- A. Check serum B12 level when patients have any of the following:
- (1) Neurological symptoms suggesting subacute combined degeneration of the cord or autonomic, optic, or peripheral neuropathy
 - (2) Abnormal signs including red, beefy tongue, paresthesias, ataxia, or loss of position or vibratory senses
 - (3) Mild cognitive impairment or dementia of mild to moderate intensity or two years or less duration
 - (4) History of abnormal laboratory results including low Hgb, high MCV, or peripheral smear showing corpuscular changes (eg, macrocytes and hypersegmented neutrophils)
 - (5) Malnutrition
- B. If serum B12 level has not been assessed in the past, it is useful to check a serum B12 level when patients have any of the following.
- (1) Behavioral and psychological symptoms of dementia
 - (2) History of gastric bypass surgery, stomach resection, terminal ileum disease or resection, or pancreatic insufficiency
 - (3) Long-term treatment with levodopa, histamine type 2 receptor blocker, or protein pump inhibitor
 - (4) Findings of leukoaraiosis on brain imaging studies
- C. If A (1) or A (2) are applicable and recent CBC is not available, check CBC. If A (1) or A (2) are applicable and CBC reveals low Hgb or high MCV, these findings suggest PA may be present.
- D. If serum B12 level is ≤ 200 pg/mL, check antiparietal cell and anti-intrinsic factor antibodies. If both are negative, then there is low likelihood that the patient has PA. If both are positive, then there is high likelihood that the patient has PA.
- E. If serum B12 level is ≤ 200 pg/mL and antiparietal cell and anti-intrinsic factor antibodies are incongruent (ie, one is positive, but the other is negative), check the serum gastrin level. If elevated, then there is high likelihood that the patient has PA.
- F. If serum B12 level is 201 to 350 pg/mL, then check plasma Hcy.
- (1) If plasma Hcy ≤ 14 μ moles/L, there is low likelihood for metabolic B12 deficiency.
 - (2) If plasma Hcy > 14 μ moles/L, there is high likelihood for metabolic B12 deficiency.
- G. Initiate vitamin B12 treatment in those cases where serum B12 levels are 200 pg/mL or less and those where serum B12 levels are 201–350 pg/mL with plasma Hcy greater than 14 μ mol/L.
- H. If anemia is severe, consider a dosage regimen similar to that made by American Regent, a manufacturer for Cobalamin Injection: 100 μ g daily for one week, then every other day for two weeks, then every third day for three weeks, then monthly thereafter, by IM or deep SC injection.* One may consider changing from parenteral to oral therapy (as in paragraph I below) after hematological abnormalities return to normal, rather than monthly vitamin B12 injections. If vitamin B12 deficiency is due to PA, supplemental B12 therapy is continued for life.
- I. If anemia is less severe or absent, other preferred dosage regimens, including cyanocobalamin 1,000 μ g PO daily, are more preferable and potentially more effective. Other common practices include cyanocobalamin 1,000 μ g IM daily for one week, then weekly for one month, then monthly thereafter.
- J. Since folate and B12 deficiencies often occur concomitantly, checking serum folate levels also may be useful whenever B12 deficiency is suspected.

Figure 4 Suggestions for vitamin B12 workup and treatment in patients with suspected neuropathology.

Notes: *Studies have reliably shown that PO cyanocobalamin therapy is another option.

Abbreviations: μ mol/L, micromoles per liter; CBC, complete blood count; Hcy, homocysteine; Hgb, hemoglobin; IM, intramuscular; MCV, mean corpuscular volume; PA, pernicious anemia; pg/mL, picograms per milliliter; PO, oral; SC, subcutaneous.

In what was described as the most severe neuropathic epidemic of modern times,⁴⁰² between 1991 and 1993 more than 50,000 Cubans developed peripheral neuropathy, associated with reduced nutrient intake of group B vitamins,^{403–409} in the

setting of strict embargos and economic deterioration.^{404,405,407,408} Although widespread distribution of group B vitamins^{404,406,407,409} and government-mandated folic acid fortification^{404,410} curbed the epidemic,^{404,406} some speculate that endemic subclinical

group B vitamin deficiencies, coupled with *Helicobacter pylori* (*H. pylori*) infections, are responsible for the higher prevalence of dementia in Cuba compared with other Caribbean countries.⁴⁰⁴ Before and after the Cuban epidemic, in 1976 and 1995, researchers^{411,412} discovered that folate deficiency and relatively high, compared with relatively low, Hcy levels in pregnant women are associated with neural tube defects (NTDs) in infants born to such women, findings that have since been replicated.^{7,18,52,355,393,413,414} Since supplemental folic acid during pregnancy decreases the risk for such abnormalities,^{415–420} in 1998 the United States Food and Drug Administration (FDA) and Health Canada required wheat and other grain products to be fortified with folic acid.^{35,58,421–423} To date, more than 50 countries have mandated folic acid fortification.^{410,420} It is largely accepted that high-dose folic acid may mask B12 deficiency. It is less clear whether or not low dose folic acid masks B12 deficiency.^{285,401} Although the United States FDA requires 140 µg of folic acid per 100 g of grain or flour,^{424,425} an amount chosen because it was considered high enough to prevent NTDs, but low enough to not mask B12 deficiency, some have advocated increasing this amount.⁴²⁶ Due to insufficient sensitivity, neither Hgb nor MCV are useful in ruling out B12-deficient dementia or SCD.^{7,13,33,58,124,289,300,371,373,378,385,427} Although the complete blood count (CBC) is part of routine diagnostic testing for dementia,³⁶⁸ additional vigilance is needed,^{20,58,381,428} especially in the era of folic acid fortification. In B12 deficiency, supplemental folic acid may protect against anemia, but not neurodegeneration,^{19,52,371} thus, the CBC alone may become even less sensitive in evaluating B12-deficient psychiatric and neurological abnormalities.

Further evaluation to rule out type A chronic atrophic gastritis, or PA,^{181,429} might be warranted because of increased risk for gastric cancer, where radiographic or endoscopic screening is useful, especially in cases of MCI and early dementia.^{15,18,33,200,201} Thus, it is useful to obtain additional testing when B12-deficient dementia occurs with anemia, macrocytosis, corpuscular changes on peripheral smear, or signs and symptoms of SCD or peripheral neuropathy.⁷ When B12-deficient dementia occurs in the absence of such findings, the decision to rule out type A chronic atrophic gastritis may be made on a case by case basis.

Although PA, or type A chronic atrophic gastritis,^{181,429} is often cited as the most common cause of low serum B12,^{7,18,19,33,49,56,58,180,201,390} emerging evidence suggests dissociation between PA and B12-deficient dementia.^{10,13,15,17–19,21,30,32,33,45,47–49,56,58,91–93,146,180,190,200,201,203,290,350,368,371,373,376,393,427–434} Thus, B12-deficient dementia may be subdivided into B12-deficient dementia with PA and B12-deficient dementia without PA. Studies support

the postulate that these two disease states are different.^{10,13–15,17,19,21,25,30,32,33,45,47–49,58,91–93,146,190,201,203,289,290,350,368,427,429,431–435} They appear to have different etiologies, pathophysiological findings, prevalences, and responses to treatment.

B12-deficient dementia with PA and B12-deficient dementia without PA have separate etiologies, whereby the former is caused by type A chronic atrophic gastritis^{181,429} and the latter is generally caused by type B (nonautoimmune) chronic atrophic gastritis, age-associated decrease in hydrochloric acid production, or decreased vitamin B12 ingestion.^{9,10,14,17,21,22,30,45,47,49,58,91,92,203,368} Type B chronic atrophic gastritis and age-associated decrease in hydrochloric acid production cause food-cobalamin malabsorption.³⁰ Less established causes of relative B12 deficiency include impairment of vitamin B12 transfer, from capillaries to CSF⁴³⁶ and from CSF to neurons,³⁸² and vitamin B12 hyperconsumption,^{54,63,340,342} inactivation,¹⁴⁶ or destruction.²⁹⁰ Type A chronic atrophic gastritis is due to antiparietal cell and anti-intrinsic factor antibodies,^{15,33,58,181,201,203,429} which occur in 90% and 55% of cases, respectively,^{7,203} causing parietal cell insufficiency and intrinsic factor dysfunction, sparing the antrum.^{33,58,201} Type B chronic atrophic gastritis is usually caused by *H. pylori* infection, involving the antrum.^{15,58,201,203} This is especially worth mentioning, because gastritis involving both body and antrum⁴³⁷ and *H. pylori* infection, confirmed by serological,^{437–439} histological,^{437,438,440} and rapid urease⁴³⁸ tests, is significantly more prevalent in individuals with MCI⁴³⁷ and AD^{438–440} compared to controls. In those with MCI, there is a correlation between serum anti-*H. pylori* immunoglobulin G concentration and degree of cognitive impairment,⁴³⁷ which may be mediated by elevated Hcy levels.⁴³⁷ One study⁴³⁸ showed *H. pylori* eradication improved both cognitive and functional measures in patients with AD. Since gastrin is secreted by antral G-cells, serum gastrin is elevated with type A,³⁷⁸ but low with type B,²¹ chronic atrophic gastritis.

B12-deficient dementia with and without PA have separate pathophysiological findings, where myeloneuropathy is characteristic and macrocytosis or anemia are present in the former,^{181,190,429} but myeloneuropathy may be rare¹⁹ and macrocytosis and anemia are often absent^{13,19,25,30,92,146,289,290,350,427,431–433} in the latter. Neuropathy associated with PA classically involves disease progression, beginning in the cervicothoracic region of the spine,^{202,441} with upper extremity findings classically occurring before lower extremity findings, followed by peripheral nerve involvement, and lastly, if at all, brain involvement.^{23,200,203} Dementia is believed to be a late and rare finding of neuropathy associated with PA.^{19,25,48,203,290}

Alternatively, dementia with B12 deficiency often occurs independently from PA.^{13,25,146,290,427,432,433}

B12-deficient dementia without PA is relatively common,^{25,146,290,427,432,433} but B12-deficient dementia with PA is considered rare,^{14,19,25,30,32,48,93,203,290,427,432} where it causes only a small fraction of B12 deficiency in demented individuals.^{14,25,30,32,93,290,427,432} PA incidence peaks late in midlife,^{181,429} while dementia incidence increases with age.²³³ Both chronic atrophic gastritis type A and B are characterized by low pepsinogen I/pepsinogen II ratios and achlorhydria,^{7,15,21,22,33,378} however, low pepsinogen I/pepsinogen II ratios occur in roughly 30% of elderly populations³⁷⁸ and PA occurs in only about 2%,^{23,428} suggesting the majority of cases with low ratios are caused by factors other than PA.

Meta-analyses or systematic reviews confirm that HHcy is a risk factor for AD,^{111,233} but they also confirm that vitamin B12 treatment of B12-deficient dementia is ineffective for improving cognitive function.^{16,348,355} Thus, B12-deficient dementia without PA and B12-deficient dementia with PA have distinct responses to supplemental B12 treatment, where it appears that moderately severe to severe dementia does not improve in the former,^{13,15,16,24,26,33,92,187,326,349,350,355,356,358,359,362} but may improve in the latter.^{185,190,199,429,434,435} The apparent dissociation of hematological findings and neurocognitive impairment provides additional support to the postulate that B12-deficient dementia with or without PA represent two separate disease states. Progressive neurocognitive decline often occurs in the presence of normal Hgb and MCV, where one or both values are normal in many individuals with B12-deficient dementia^{13,33,124,188,200,433} or neurological abnormalities.^{18,20,124,180,188,373} When anemia is absent, Hgb is indirectly proportional to cognitive function,^{18,19,200} and when present, it is an inconsistent risk factor for cognitive dysfunction.^{92,430} Neurological impairment occurring concomitantly with anemia is more likely to improve with supplemental B12 therapy in those cases where the anemia is more severe and less likely to improve where the anemia is less severe.^{180,393} With supplemental B12 therapy, increased reticulocytosis occurs within one week,^{376,393} but neurological improvement may require six months or longer.^{180,393} Vitamin B12 supplementation reverses hematological abnormalities in almost all patients,^{15,33,185,362} may improve neurological abnormalities in roughly one-half,^{15,33,185} and arrests or reverses dementia in only very few.^{13,15,16,24,26,33,92,187,326,347,349,350,355,361,362}

In B12 deficiency dementia with versus without PA, there appear to be different manifestations, need for further workup, and responses to treatment. Therefore, when findings include low serum B12 or elevated Hcy, it is useful to rule out PA. In the workup for type A chronic atrophic gastritis, assessment of

antiparietal cell and anti-intrinsic factor antibodies is an initial option.^{32,181,368,429} Since the former are sensitive, occurring in up to 10% of elderly populations,⁵⁶ and the latter are specific, the absence of antiparietal cell antibodies suggests low likelihood,⁷ while the presence of anti-intrinsic factor antibodies suggests high likelihood,⁷ for type A chronic atrophic gastritis. If antiparietal cell antibodies are present and anti-intrinsic factor antibodies are absent, assessment of serum gastrin levels is a further option.^{7,33} Type A and B chronic atrophic gastritis also can be distinguished by endoscopy with mucosal surface biopsy.^{7,181,429} Although Schilling's test has been largely supplanted by the aforementioned tests,^{203,368} it may be useful if such tests are unremarkable or equivocal.⁴³⁴ However, decreased vitamin B12 ingestion^{9,47} and food-cobalamin malabsorption^{9,45,49} are common causes of low serum B12 in elderly populations, and Schilling's test is most often negative (ie, step one results are normal) in these conditions.^{25,33,93,203}

When vitamin B12 deficiency is found in dementia, is dementia of the Alzheimer's type a compatible diagnosis?

AD is diagnosed by tissue pathology. According to the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Text Revision (DSM-IV-TR), DAT is essentially a diagnosis of exclusion, after other causes of dementia, including B12 deficiency, have been ruled out, thus dichotomizing dementia due to B12 deficiency and DAT. Although two studies^{359,442} support this dichotomy, most studies^{13,26,82,222,333,336,382} suggest DAT occurs with or without B12 deficiency. When MCI and various subtypes of dementia are compared with one another, similar concentrations of serum B12 and Hcy are observed.^{276,284,443} When B12-deficient dementia is compared to other subtypes of dementia, similar rates of decline are observed across almost all measured outcomes.^{336,350,444} Nonetheless, there is likely a DAT subgroup, having low serum B12^{427,445} and perhaps elevated platelet monoamine oxidase (MAO) activity.²⁹ Since B12 deficiency dementia with PA may arrest or reverse with treatment^{190,429,434} and B12 deficiency dementia without PA is usually progressively neurodegenerative despite treatment, the presence of PA would suggest dementia due to B12 deficiency, while its absence would suggest DAT.⁴⁴⁶

Treatment

Based on the benefit-to-risk ratio in treatment of dementia, if vitamin B12 deficiency is determined, should supplemental B12 be initiated?

Vitamin B12 treatment is considered one of the safest medical treatments available.⁴⁴⁷ Although severe adverse events are

very rare, those reported include anaphylactic shock and death with administration of parenteral vitamin B12, hypokalemia with sudden death in conditions of severe anemia, and severe and sudden optic atrophy in those with Leber's hereditary optic neuropathy.⁴⁴⁸ Other adverse events include polycythemia, thrombosis, pruritus, rash, skin eruptions⁴⁴⁹ congestive heart failure, diarrhea, edema, and swelling. One randomized controlled trial (RCT)³⁴⁷ found a greater prevalence of depression in the treatment group, who received high-dose pyridoxine, folate, and vitamin B12, than in the placebo group.

Although no overdosage has been reported, in treating hypovitaminosis B12 with supplemental B12, there is a fine line between help and harm. The serum cobalamin-plasma Hcy concentration-response curve appears curvilinear.¹⁷ As serum cobalamin increases from the lowest detectable level to 950 pg/mL, plasma Hcy decreases, but as serum cobalamin further increases from 950 pg/mL to 1,350 pg/mL, plasma Hcy begins to rise. One cohort study,³⁸⁶ found a dose-dependent association between increasing serum B12 levels and incidence of coronary artery disease (CAD) and mortality, where each 100 pg/mL increase in serum cobalamin was associated with a 10% increased incidence for such events. One RCT⁴⁵⁰ found that low-dose pyridoxine, folic acid, and vitamin B12 treatment decreased the risk for stroke, CAD events, and death, while moderately high dose pyridoxine, folic acid, and vitamin B12 treatment did not. Although the earliest two^{451,452} of eight other RCTs showed combined pyridoxine, folic acid, and vitamin B12 treatment decreased morbidity in individuals with or at risk for CAD, the later six⁴⁵³⁻⁴⁵⁸ failed to validate these findings, and four^{454,456-458} of the six showed treatment potentially increased the risk for harm. One of these RCTs⁴⁵⁵ was prematurely terminated due to potential risk for harm demonstrated by group B vitamin treatment.⁴⁵⁴ Therefore, some authors^{386,454} suggest supplemental B12 should not be administered unless B12 deficiency is present, and when present, only enough supplemental B12 should be administered to correct the deficiency. Parenthetically, meta-analyses of folic acid treatment, aimed at lowering HHcy, show mixed results in terms of whether or not treatment decreases the risk for stroke.^{459,460} Since most prospective studies show supplemental B12 does not improve cognitive function or prevent dementia in cognitively intact individuals,^{306,354,360} including those with hypovitaminosis B12^{351,353} or HHcy,³⁵⁷ it is not recommended⁴⁰¹ for these purposes.

HHcy increases the risks for birth defects, cognitive dysfunction, dementia, cerebrovascular disease, stroke, CAD, MI, peripheral vascular disease, venous thrombosis,

osteoporosis, hip fractures, and death.^{21,39,279,386,461-468} Benefits of treating B12 deficiency include lowering Hcy levels,^{19,21,47,135,198,326,347,351,357,360-362,397,469} lowering A β peptide levels,⁴⁷⁰ decreasing tau hyperphosphorylation,¹³² anti-inflammation,⁴⁷¹ neuroprotection,⁴⁷¹ reduction of MAO activity,²⁹ and causing the BBB to be less leaky.⁴⁶⁹

It may not be feasible to perform a meta-analysis on studies that have ascertained whether or not vitamin B12 treatment is associated with improved cognitive function or prevention of dementia due to the heterogeneity of these studies (Table 2). Published studies include multiple designs (eg, case-control, other retrospective studies, RCTs, and other prospective studies), subjects (eg, those who are healthy, ill, community-dwelling, residents of tertiary care facilities, with and without cognitive dysfunction, and with and without vitamin B12 deficiency), interventions, including vitamin B12 alone or in combination with other drugs or dietary supplements, type of vitamin B12 administered, and route of administration.

Traditional recommendations of assessing for and, when found, treating B12-deficient dementia are supported by case-control studies,^{350,359} retrospective series,^{49,180,188,435,472} and case reports,^{190,429,434} where B12 deficiency was specifically caused by PA. Evidence supporting these recommendations appears to diminish with many RCTs,^{16,185,198,298,306,326,347,348,351-353,355,357,360,361,364,473,474} clinical trials (CTs),^{13,24,185,187,199,346,356,362,397,469} and other prospective studies^{26,92,349,358,475} (Tables 3a and b). Twelve^{16,306,326,347,351-353,355,357,360,361,364} of 16 RCTs found supplemental B12 therapy was of no benefit for improving cognitive function. Four^{185,198,473,474} of 16 RCTs found supplemental B12 therapy was beneficial for improving cognitive function; however, subjects in these studies included nondemented, healthy adult women,⁴⁷³ individuals with schizophrenia and HHcy,¹⁹⁸ and individuals with dementia, irrespective of serum B12 levels, treated with multiple vitamins and other dietary supplements.⁴⁷⁴ The design of one of these studies¹⁸⁵ did not include a separate control group. In all other RCTs of individuals with hypovitaminosis B12,^{16,351,353,355} HHcy,³⁵⁷ HMMA,³⁵² dementia,^{16,326,347,355,361} or at risk for dementia,^{326,348,352,360,364} vitamin B12 treatment group results were no better than placebo group results. Nonetheless, the results from the RCTs may not generalize to all patients. Five of these^{306,351,353,357,360} did not examine subjects with MCI or dementia, three^{16,326,355} may have included subjects in various dementia stages, and six appear to have enrolled subjects, with either normal^{347,361} or irrespective^{306,326,360,364} of baseline folate and vitamin B12 statuses.

Although few included control groups,^{198,199,473,474} CTs and other prospective studies with positive

Table 2 Examples of multiple binary variables in studies examining efficacy of B12 treatment on cognition

No	Time course	Type of study	Absence/presence of cognitive dysfunction	B12 status*	Study results; author, year
1	Retrospective and cross-sectional	Not case control	Absent	Unknown	Positive; La Rue, 1997
2		Not case control	Absent	Low	Positive; Healton, 1991 ¹
3		Not case control	Present	Low	Positive; Lindenbaum, 1988 El Otmari, 2008 ² Fox, 1975 ³ Healton, 1991 ¹
4		Case control	Absent	Normal	Negative; Lin, 2008 ⁴
5		Case control	Present	Low	Negative; Eastley, 2000 ⁵
6		Case control	Present	Low	Positive; Eastley, 2000 ⁵ Osimani, 2005 ⁶
7	Prospective	Not randomized controlled trial	Absent	Low	Positive; van Asselt, 2001
8		Not randomized controlled trial	Present	Unknown	Positive; Ikeda, 1992 ⁷
9		Not randomized controlled trial	Present	Normal	Positive; Lehmann, 2003 ⁸
10		Not randomized controlled trial	Present	Low	Negative; Carmel, 1995 Crystal, 1994 ⁹ Cunha, 1990 ¹⁰ Kwok, 2008 ¹¹ Teunisse, 1996 ¹² van Dyck, 2008 ¹³
11		Not randomized controlled trial	Present	Low	Positive; Abyad, 2002 ¹⁴ Cunha, 1995 ¹⁵ Kalita, 2008 Kwok, 2008 ¹¹ Martin, 1992 ¹⁶ Nilsson, 2001 ¹⁷
12		Randomized controlled trial	Absent	Unknown	Negative; Stott, 2005 ¹⁸ Wolters, 2005
13		Randomized controlled trial	Absent	Unknown	Positive; Bryan, 2002
14		Randomized controlled trial	Absent	Normal	Positive; Levine, 2006 ¹⁹
15		Randomized controlled trial	Absent	Low	Negative; Eussen, 2006 Kwok, 1998 McMahon, 2006
16		Randomized controlled trial	Present	Unknown	Negative; van Uffelen, 2008
17		Randomized controlled trial	Present	Unknown	Positive; Remington, 2009 ²⁰
18		Randomized controlled trial	Present	Normal	Negative; Aisen, 2008 Clarke, 2003 ²¹ Sun, 2007
19		Randomized controlled trial	Present	Low	Negative; Clarke, 2003 ²¹ Fourniere, 1997 Hvas, 2004 Seal, 2002
20		Randomized controlled trial	Present	Low	Positive; Bolaman, 2003 ²²

Notes: 1, Subjects had nervous system involvement; 2, N = 1; 3, N = 1; 4, sample consisted of asymptomatic nursing home males; 5, results were negative for subjects with dementia, and positive for those with mild cognitive impairment; 6, baseline neuropsychiatric tests determined those who did not improve from those who improved; 7, B12 status was not a major factor mentioned in the methods or results sections, treatment consisted of intravenous mecobalamin; 8, subjects had normal vitamin B12, hyperhomocysteinemia, and mild cognitive impairment at baseline, and there were no subjects who progressed to dementia; 9, N = 3; 10, N = 13; 11, delirium improved, but dementia did not improve; 12, N = 26; 13, N = 28; 14, shorter duration of cognitive dysfunction was associated with greater response to B12 treatment; 15, outcome was obtained in 19/46 patients, 16/19 worsened, and 3/16 improved with B12 therapy, those who improved had mild dementia of less than two years duration; 16, individuals who were symptomatic less than 12 months improved with B12 therapy; 17, those with mild-moderate dementia and those with hyperhomocysteinemia improved with supplemental B12 therapy; 18, subjects were elders with ischemic vascular disease; 19, subjects had schizophrenia; 20, the dietary supplement also contained N-acetylcysteine, S-adenosylmethionine, and multiple other ingredients; 21, the sample presumably included those with normal and low serum B12; 22, all subjects had pernicious anemia, oral was compared with intramuscular cyanocobalamin. *Binary variables include B12 status: known versus unknown, and known-normal versus known-low.

results^{24,185,187,198,199,346,356,358,397,469,473–475} show cognitive improvement may be associated with five factors: Hcy state,^{37,57,200,358,397} disease duration,^{18,24,30,180,346,356} disease intensity,^{24,180,350,358,359} and treatment type,^{474,475} as shown in Table 4.^{17,24,37,58,146,180,290,306,346,350,351,353,356–359,361,473–495} The fifth factor, and perhaps the

most striking, is whether or not the B12 deficiency-associated cognitive dysfunction is due to PA. If so, then these individuals may respond remarkably well to supplemental B12 treatment, regardless of the severity of the dementia.^{185,190,429,434} Parenthetically, all RCTs show supplemental folic acid is no better than

Table 3a Studies showing vitamin B12 treatment is not associated with improved cognitive function or prevention of dementia

No	Principal author, Year	Study design	Type of sample	N	Duration	Major measures	Exposures/treatments	Major results/outcomes
1	Aisen, 2008	A multicenter, randomized, double-blind, controlled clinical trial	Patients with mild to moderate AD and normal folic acid, vitamin B12, and Hcy levels	409	18 months	ADAS-cog	High-dose folic acid, vitamin B6, and vitamin B12 treatment	Vitamin treatment was not beneficial on the rate of change in the ADAS-cog score
2	Carmel, 1995	Prospective investigation	Demented and nondemented patients with low serum B12	16	NA	Neurological examination, EEG, visual evoked potentials, somatosensory potentials, dUST, plasma Hcy, serum MMA	Vitamin B12 treatment Neurological examination, EEG, visual evoked potentials, somatosensory potentials, dUST, plasma Hcy, serum MMA	50% of patients had abnormal dUST, 44% had increased plasma Hcy and/or MMA, 73% had mild neurological abnormalities (primarily neuropathies), 75% had EEG abnormalities, 77% had abnormal visual evoked potentials, 33% had abnormal somatosensory potentials Although B12 treatment improved most abnormalities, it did not improve cognitive function in the 13 demented patients Treatment did not affect cognitive function
3	Clarke, 2003	Randomized, double-blind, placebo-controlled trial	Individuals with MCI or dementia, irrespective of baseline serum B12 and plasma Hcy levels	149	3 months	Cognitive function assessment, plasma Hcy, serum folate, serum B12, urine 11-dehydrothromboxane B2 (a marker of platelet activation) and 8-epiprostaglandin F2alpha (a marker of reactive oxygen species)	Low-dose aspirin treatment, folic acid and B12 treatment, vitamin E and vitamin C treatment, placebo	Treatment did not affect cognitive function
4	Crystal, 1994	Cohort	Ambulatory, nondemented, healthy elderly	79 of 388 individuals developed dementia	60 months	Specific neuropsychological tests, serum B12	Vitamin B12 treatment	22 of 388 patients had low serum B12 levels; 3 of these became demented. 57 of 388 individuals had higher B12 levels. AD incidence among the low B12 group was 4.5% compared with 7.5% in the higher B12 group. Mean B12 level at time of diagnosis in subjects who developed AD was 551 pg/mL. There was no evidence of hematologic abnormalities among the 22 subjects with low B12. Of the 3 low B12 patients who became demented, none responded to IM B12 Mental status examination did not improve, and most cases demonstrated persistent cognitive deterioration
5	Cunha, 1990	Prospective investigation	Demented elderly outpatients	13 of 110 patients had low serum B12	NA	Mental status examination	Vitamin B12 treatment	There were no cases of arrestable or reversible dementia. Demented patients with low serum B12 did no better with B12 treatment than demented controls without low serum B12
6	Eastley, 2000*	Case-control	Mildly cognitive impaired or demented outpatients with low serum B12	88	NA	Specific neuropsychological tests, Hgb, MCV, serum B12	Vitamin B12 treatment	There were no cases of arrestable or reversible dementia. Demented patients with low serum B12 did no better with B12 treatment than demented controls without low serum B12

7	Eussen, 2006	Randomized, double-blind, placebo-controlled trial	Elders with mild vitamin B12 deficiency	195	6 months	Specific neuropsychological tests	Vitamin B12 treatment, folic acid and vitamin B12 treatment, or placebo	Neither treatment group demonstrated cognitive improvement. The placebo group demonstrated greater memory improvement than the vitamin B12-alone group
8	Fourniere, 1997	Randomized, placebo-controlled trial	Demented individuals with low serum B12	NA	NA	NA	Vitamin B12 treatment	No improvement in cognitive function
9	Hvas, 2004	Randomized, placebo-controlled trial	Individuals with elevated plasma MMA, not previously treated with vitamin B12, many of whom had cognitive impairment at baseline	140	3 months	Cognitive function assessed by the CAMCOG, MMSE, and a 12-words learning test, depressive symptoms evaluated by the MDI	Vitamin B12	Treatment group did no better than placebo group on any of the measured outcomes of cognitive function or depressive symptoms
10	Kwok, 1998	Randomized clinical trial	Apparently healthy elders with low serum B12	50	4 months	MMSE, specific neuropsychological tests, serum B12, serum MMA	Vitamin B12 treatment or no intervention	Individuals treated with IM B12 performed more poorly on motor function scores compared with the control group Individuals treated with intramuscular B12 demonstrated improvement in performance IQ, but the improvement was not statistically significant compared with the control group Overall, individuals treated with intramuscular B12 showed no improvement in cognitive function compared with the control group
11	Kwok, 2008*	Clinical trial	Mild to moderately demented patients with low serum B12	30	9 months	MMSE, specific neuropsychological tests, serum B12	Vitamin B12 treatment	There were no significant changes in cognitive function or behavioral symptoms
12	Lin, 2008	Case-control	Asymptomatic nursing home elderly males, excluding those with renal insufficiency or low serum B12	419		Vitamin B12 supplementation status, MMSE, GDS, serum B12	Vitamin B12 supplementation	Cognitive impairment and depression prevalences were similar in those taking and not taking B12 supplements. MMSE and GDS were similar between those taking and not taking B12 supplements Hcy lowering therapy did not improve cognitive function in elders with Hcy
13	McMahon, 2006	Randomized, double-blind, placebo-controlled trial	Healthy elders with plasma Hcy at least 13 μmol per liter	276	24 months	Plasma Hcy	Folate, vitamin B6, and vitamin B12 treatment	No improvement in cognitive function
14	Seal, 2002	Randomized, placebo-controlled trial	Demented individuals with low serum B12	NA	NA	NA	Vitamin B12 treatment	No improvement in cognitive function
15	Stott, 2005	Randomized, double-blind, placebo-controlled trial	Elders with ischemic vascular disease	185	12 months	Specific neuropsychological tests, plasma Hcy	Folic acid and vitamin B12 treatment, riboflavin treatment, or vitamin B6 treatment	Folic acid and vitamin B12 did not improve cognitive function in elders with ischemic vascular disease

(Continued)

Table 3a (Continued)

No	Principal Author, Year	Study design	Type of sample	N	Duration	Major measures	Exposures/ treatments	Major results/outcomes
16	Sun, 2007	Randomized, double-blind, placebo-controlled trial	Elders with mild to moderate AD and normal serum folate and B12	89	6 months	ADAS-cog, ADL evaluation, serum folate, serum B12, plasma Hcy	AChE I and mecobalamin plus multivitamin containing vitamin B6, folic acid, other vitamins, and iron or AChE I and placebo	Supplemental mecobalamin plus multivitamin was no different than placebo in ADAS-cog scores and ADL evaluations
17	Teunisse, 1996	Prospective investigation	Outpatients with dementia	26 of 170 had low serum B12	6 months	Cognitive function, dementia severity, ADLs, behavioral disturbances, caregiver burden, serum B12	Vitamin B12 treatment	B12 treatment did not improve functioning in demented patients with low serum B12. The rate of dementia deterioration in patients with low serum B12 was the same as those with AD
18	van Dyck, 2008	Clinical trial with blinded raters and a comparison group	Elderly, demented, nursing home residents with low serum B12	28 in the low serum B12 group, 28 in the comparison group with normal serum B12	2 months	DRS, BPRS, GDS, serum B12	Vitamin B12 treatment	B12 treatment did not produce significant effects on cognitive or psychiatric variables B12 treatment produced significant improvement in hematologic and metabolic parameters
19	van Uffelen, 2008	Randomized, placebo-controlled trial	Community-dwelling elders with MCI	152	12 months	Specific neuropsychological tests, quality of life measurements	Moderate intensity walking program versus a low intensity placebo activity; combined vitamin B6, folic acid, and vitamin B12 treatment versus placebo	Those in the moderate intensity walking group showed small improvements or trends in a few of the subscales of neuropsychological tests. Those in the vitamin treatment group were no different than placebo across cognitive function measurements
20	Wolters, 2005	Randomized, double-blind, placebo-controlled trial	Healthy, free-living, well-nourished, elderly women without dementia	220	6 months	Specific neuropsychological tests, RBC folate, RBC functional B6 activity, serum folate, serum B12, plasma Hcy, serum MMA	Multivitamin supplementation	Multivitamin supplementation did not improve cognitive function

Table 3b Studies showing vitamin B12 treatment is associated with improved cognitive function or prevention of dementia

No	Principal Author, Year	Study design	Type of sample	N	Duration	Major measures	Exposures	Major results/outcomes
1	Abyad, 2002	Single-blind clinical trial	Cognitively impaired nursing home residents and geriatric outpatients with low serum B12	62	12 months	MMSE, clock drawing test, caregiver interviews	Vitamin B12 treatment	40 of 56 patients completing the study showed cognitive improvement. The shorter the duration of cognitive dysfunction, the greater the response to parenteral B12 therapy
2	Bolaman, 2003	Randomized clinical trial	Patients 16 years of age or older with megaloblastic anemia due to B12 deficiency	60	3 months	WBC count, reticulocyte count, Hgb, MCV, platelet count, serum B12, neurological evaluation, including MMSE and assessment of vibration, light touch, and pain Baseline neurological findings included recall and/or concentration deficit in 7 patients, loss of the sense of light touch and pain in 9 patients, and loss of the sense of vibration in 5 patients	PO versus IM vitamin B12	In both groups, serum B12 increased. In both groups, Hgb, WBC count, and platelet count increased, MCV decreased, and reticulocytosis was observed. Neurologic improvement was detected in 7 of 9 patients in the PO B12 group and 9 of 12 patients in the IM B12 group
3	Bryan, 2002	Randomized clinical trial	Nondemented, healthy adult women	211	1 month	Food frequency questionnaire, specific neuropsychological tests, subjective mood assessment	Usual dietary intake of B-complex vitamins, combined vitamin 6, folate, and vitamin B12 treatment, or placebo	Dietary intake status was associated with speed of processing, recall, recognition, and verbal ability.
4	Cunha, 1995	Clinical trial	Elderly demented outpatients	46 of 181 individuals had low serum B12	3 to 24 months	MMSE, serum B12	Vitamin B12 treatment	Supplemental B12 slightly improved memory, but had no effect on mood Outcome was obtained in 19 of the 46 patients with low serum B12. 16 of the 19 cognitively declined; 3 of the 19 cognitively improved. The 3 that improved had mild dementia of less than 2 years duration
5	Eastley, 2000*	Case-control	Mildly cognitive impaired or demented outpatients with low serum B12	88	NA	Specific neuropsychological tests, Hgb, MCV, serum B12	Vitamin B12 treatment	Patients with low serum B12 and MCI, treated with B12, improved in verbal fluency compared to controls
6	El Otmani, 2008	Case series	Patients with B12 deficiency involving the nervous system	27	NA	Neurological examination, serum B12	Vitamin B12 treatment	Of 27 cases, 1 had dementia, which improved with B12 treatment Of the remaining cases, 12 improved neurologically with B12 treatment
7	Fox, 1975	Case series	Patients with dementia	40	NA	Various laboratory and radiological tests, including computerized axial tomography	Vitamin B12 treatment	A patient with dementia with PA demonstrated marked improvement with B12 treatment
8	Healton, 1991	Case series	Patients with B12 deficiency involving the nervous system	143	204 months	Neurologic impairment quantitative severity score, Hct, MCV, neutrophil count, platelet count, serum B12	Vitamin B12 treatment	Of 121 episodes of low serum B12, 64 had a partial response and 57 had a complete response

(Continued)

Table 3b (Continued)

No	Principal Author, Year	Study design	Type of sample	N	Duration	Major measures	Exposures	Major results/outcomes
9	Ikedo, 1992	Prospective investigation	Individuals with AD	10	NA	Intellectual function rating scales, serum B12, serum B12 unsaturated binding capacities, CSF B12	Vitamin B12 treatment	IV mecobalamin improved memory, emotional function, and interpersonal communication. Improvement of interpersonal communication and memory occurred after a serum B12 threshold was maintained for sufficient time and at relatively higher CSF B12 levels
10	Kalita, 2008	Clinical trial of patients, compared with a representative control group	Patients with low serum B12 and/or megaloblastic bone marrow	36	3 months	Neurological examination, MMSE, craniospinal MRI, cognitive evoked potentials, WBC count, RBC count, RBC indices, Hgb, serum chemistry, HIV titer, thyroid profile, antiparietal cell antibodies	Vitamin B12 treatment	The presenting syndrome was myelopathy in 8, cognitive in 1, myeloneuropathy in 10, myelocognitive in 8, and myeloneurocognitive in 9 patients. MMSE was abnormal in 17 patients. Cranial MRI demonstrated cortical atrophy in 1 and multiple white matter hyperintensities in 3 patients. P3 latency was either prolonged or unrecordable in 15 patients compared to controls. Both MMSE and P3 latency improved
11	Kwok, 2008*	Clinical trial	Mild to moderately demented patients with low serum B12	30	9 months	MMSE, specific neuropsychological test, serum B12	Vitamin B12 treatment	DRS significantly decreased between baseline and 9 months
12	La Rue, 1997	Retrospective analysis	Community-dwelling elders without MCI or dementia	137	72 months	Specific neuropsychological tests, current and past intake of dietary proteins, dietary vitamins, and supplements	Dietary and supplemental vitamin B12 intake	Higher past B12 intake was related to better performance on recall, visuospatial, or abstraction testing
13	Lehmann, 2003	Clinical trial	Mildly cognitive impaired patients with HHcy, normal serum B12 and normal serum folate	30	9 months	Plasma albumin, plasma Hcy, serum B12, serum folate, CSF albumin, CSF tau protein, Baseline CSF-to-plasma albumin ratio was higher in patients compared to a control group.	Combined vitamin B6, folic acid, and vitamin B12 treatment	Reduction of the CSF-to-plasma albumin ratio CSF tau protein levels did not significantly change, although there was a numeric decline. None of the patients progressed to dementia during the treatment period
14	Levine, 2006	Randomized, double-blind, placebo controlled	Individuals with HHcy and schizophrenia	42	3 months	Neuropsychological testing, including the WCST, PANSS	Vitamin B12 treatment	Neuropsychological testing, in general, and the WCST, in particular, improved in the treatment group. PANSS declined in the treatment group
15	Lindenbaum, 1988	Case series	Patients with B12 deficiency involving the nervous system	141	NA	Neurologic impairment quantitative severity score, Hct, MCV, neutrophil count, platelet count, serum B12, plasma Hcy, serum MMA	Vitamin B12 treatment	Of 121 episodes of low serum B12, all patients responded to B12 treatment, except one patient who died All patients with neuropsychiatric findings associated with low serum B12 with HHcy or MMA responded to B12 treatment

16	Martin, 1992	Clinical trial	Cognitively impaired elderly with low serum B12	18	6 months	MDRS	Vitamin B12 treatment	11 patients improved cognitively with IM cyanocobalamin. Patients symptomatic for less than 12 months gained an average of 20 points on the MDRS; patients symptomatic greater than 12 months lost an average of three points Patients with mild-moderate dementia and elevated plasma Hcy clinically improved, with increased test scores, while severely demented patients and those with normal plasma Hcy levels did not clinically improve Twelve patients improved with treatment; seven patients did not improve with treatment. Baseline neuropsychological tests distinguished those who improved from those who did not improve
17	Nilsson, 2001	Prospective investigation	Demented elderly	33	NA	MMSE, cognitive testing for memory and attention, serum B12, blood folate, plasma Hcy, serum MMA	Folic acid and vitamin B12 treatment	Patients with mild-moderate dementia and elevated plasma Hcy clinically improved, with increased test scores, while severely demented patients and those with normal plasma Hcy levels did not clinically improve
18	Osimani, 2005	Case-control	Demented patients with low serum B12	19	12 months	Specific neuropsychological tests serum B12	Vitamin B12 treatment	Twelve patients improved with treatment; seven patients did not improve with treatment. Baseline neuropsychological tests distinguished those who improved from those who did not improve
19	Remington, 2009	Randomized, placebo-controlled	Individuals institutionalized with moderate to severe AD	12	9 months	Cognitive and functional rating scales, including DRS, CDT, NPI, and ADCS-ADL	A dietary supplement containing folic acid, vitamin B12, alpha-tocopherol, SAM, N-acetyl cysteine, and acetyl-L-carnitine	Treatment group demonstrated a delay in decline in the DRS and CDT, 30% improvement in the NPI, and maintenance of performance in the ADCS-ADL
20	van Asselt, 2001	Single-blind, placebo-controlled clinical trial	Healthy, cognitively intact, community-dwelling elders with low serum B12	16	6 months	Serum B12, plasma Hcy, serum MMA, quantitative EEG, specific neuropsychological tests	1 month placebo treatment, followed by 5 months vitamin B12 treatment	After B12 treatment, serum B12 increased and plasma Hcy and serum MMA decreased. Quantitative EEG showed more fast activity and less slow activity, which was associated with improved performance on the Verbal Word Learning Test, Verbal Fluency, and Similarities. Psychometric test performance improved on the Verbal Word Learning Test, Verbal Fluency, and Similarities. Lower plasma Hcy concentrations were related to increased fast activity on quantitative EEG and improved performance on the Verbal Word Learning Test and Similarities.

Abbreviations: Abeta40, amyloid beta-protein ending in Val-40; Abeta42, amyloid beta-protein ending in Ala-42; AChE, acetylcholinesterase inhibitor; AD, Alzheimer's disease; ADAS-cog, Alzheimer Disease Assessment Scale – cognitive subscale; ADCS-ADL, Alzheimer Disease Cooperative Study-Activities of Daily Living; ADL, activity of daily living; BPRS, Brief Psychiatric Rating Scale; CAMCOG, Cambridge Cognitive Examination; CDT, Clock Drawing Test; CSF, cerebrospinal fluid; DRS, Dementia Rating Scale; DUST, deoxyuridine suppression test; EEG, electroencephalogram; GDS, Geriatric Depression Scale; Hct, hematocrit; Hcy, homocysteine; Hgb, hemoglobin; HHcy, hyperhomocysteinemia; HIV, Human Immunodeficiency Virus; HMMA, hypermethylmalonic acidemia; IM, intramuscular; IV, intravenous; MCI, mild cognitive impairment; MCV, mean corpuscular volume; MDI, Major Depression Inventory; MDRS, Mattis Dementia Rating Scale; MMA, methylmalonic acid; MMSE, Mini-Mental State Examination; MRI, magnetic resonance image; NA, not applicable or available; NPI, Neuropsychiatric Inventory; PA, pernicious anemia; PANSS, Positive and Negative Syndrome Scale; PO, oral; RBC, red blood cell; WCST, Wisconsin Card Sorting Test. *Study appears in two areas of table based on outcomes.

placebo at improving cognitive function in individuals with dementia.^{348,355,361,496}

BPSD include symptoms and signs of disturbed behavior, mood, psychomotor activity, and thought content (eg, delusions and hallucinations) in patients with dementia. Exogenous SAM improves depression in patients with PD⁴⁸¹ and other conditions.^{494,495} In individuals with AD, those with HHcy have increased EEG slow wave activity (ie, increased theta and delta waves) compared to those without HHcy.^{184,257} In nondemented individuals, lowering plasma Hcy levels with vitamin B12 treatment reverses such EEG findings.^{184,397} Accordingly, prospective studies of individuals with B12-deficient dementia show patients with delirium¹⁸⁷ or psychoses³⁵⁹ improve with vitamin B12 supplementation. In demented individuals, prospective studies show vitamin B12 supplementation enhances vigilance when combined with bright light therapy,⁴⁹⁷ improves mood disturbances,⁴⁷⁵ but may worsen motor performance.³⁵³ Although not a consistent finding,^{187,362} supplemental B12 in B12-deficient dementia may be beneficial for some patients with BPSD.^{359,475} Independent from serum folic acid and B12 levels, an association was found between HHcy and schizophrenia;⁴⁹⁸ in spite of the HHcy association being independent from group B vitamins in that study, another study¹⁹⁸ showed combined pyridoxine, folic acid and vitamin B12 treatment in individuals with HHcy and schizophrenia improved positive symptoms, negative symptoms, and cognitive function. Depression, mania, psychoses, and delirium associated with PA or hypovitaminosis B12 may improve with supplemental B12 treatment.^{181–184,190,191}

Considering the potential risks for adverse events, added cost, and added administration, and the potential benefit for arresting or reversing dementia, if B12 deficiency is determined, it should be treated. Vitamin B12 treatment should be initiated in those cases where serum B12 levels are 200 pg/mL or less and those where serum B12 levels are 201–350 pg/mL with plasma Hcy greater than 14 µmol/L.³²⁰ Duration of therapy is based on etiology and other factors, with an optimum serum B12 not being more than 950 pg/mL. In cases of PA, treatment is continued for life. In cases of dietary deficiency, low vitamin B12 in dementia normalizes with dietary correction.¹⁴ Possible explanations on why dementia does not reverse or arrest with supplemental B12 include treatment being an ineffective form,^{146,290} initiation outside of a time course window,^{30,346,356} or dementia not solely being caused by PA or low serum B12.^{13,26,82,222,333,336,359,442}

How is vitamin B12 deficiency treated?

Since bacteria produce natural forms of vitamin B12, which humans ingest by consuming animal products,^{19,58,201} strict vegetarians may require oral (PO) vitamin B12 supplementation.⁵⁸ The recommended daily allowance (RDA) is 2.4 µg daily.⁴⁹⁹ Supplemental forms of vitamin B12 include cyanocobalamin, used in the United States, hydroxocobalamin, used in Europe, and mecobalamin, used in Asia.^{7,361,475} Mecobalamin is a synthetic form of methylcobalamin, which is the type of cobalamin utilized intracellularly. Although it may be advantageous in cognitive improvement or protection,⁴⁷⁵ it has not been compared with cyanocobalamin or hydroxocobalamin in clinical trials, and it may not be readily available in many western countries. Routes for cyanocobalamin administration include PO, sublingual (SL), intranasal (IN), intramuscular (IM), and subcutaneous (SC). Although the intravenous (IV) route has been used in patients with renal failure,^{500,501} this route is not recommended for general use.⁴⁴⁸ Compared to PO cyanocobalamin, IM and IV cyanocobalamin potentially pose greater risks for anaphylaxis. Up to 98% of an IM dose is lost in the urine, and even more is lost with an IV dose.^{387,448} Alternatively, 1% of PO cyanocobalamin is absorbed, throughout the gastrointestinal tract, by passive diffusion, independent of intrinsic factor.^{18,52,201,368,387} Thus, 10 µg will be absorbed from a 1,000 µg dose, well above the RDA,^{18,201,502} this effect is seen even in those with PA, prior gastrectomies, or diseases of the terminal ileum.^{52,368,387,503} Most physicians favor IM over PO routes,^{20,23,31,203,504} while most patients favor PO over IM routes.^{503,505} Patient preference is supported by a CT⁹¹ and RCTs^{31,34,185,392} showing PO cyanocobalamin provides equal, if not greater, serum B12 therapeutic levels. Since PO cyanocobalamin is as effective as IM cyanocobalamin in treating B12 deficiency, and its complications, it may be the preferred route of administration, unless there is concern regarding PO cyanocobalamin adherence, such as dysphagia.^{19,200,376,505} Variance exists in recommendations for dose and administration frequency: common practices include cyanocobalamin 1,000 µg PO daily^{203,503,505} or cyanocobalamin 1,000 µg IM daily for one week, then weekly for one month, then monthly thereafter.^{23,185,200,203,346,356,387,393} Alternative regimens are recommended for those with manifestations of severe PA.⁴⁴⁸

Conclusions

Although this paper represents a synopsis of our current understanding between HHcy and hypovitaminosis B12 and MCI or dementia, some investigations and reviews reveal

Table 4 Factors associated with cognitive improvement in B12 supplementation of B12-deficient dementia**Etiology**

- Treatment of B12 deficiency caused by pernicious anemia generally is associated with cognitive improvement.
- Treatment of B12 deficiency caused by etiologies other than pernicious anemia generally is not associated with cognitive improvement.

Homocysteine state

- Presence of hyperhomocysteinemia at treatment onset is associated with improvement, whereas absence is not.^{37,358}

Disease duration

- Duration of disease two years or less is associated with improvement, whereas duration of disease more than two years is not.^{24,180,346,356}

Disease intensity

- Mild cognitive impairment and mild to moderate dementia are associated with improvement, whereas moderately severe to severe dementia is not.^{24,180,350,358,359}
- Four^{306,351,353,357} of five^{306,351,353,357,473} prospective studies, in individuals without cognitive dysfunction, showed supplemental B12 does not improve cognitive function, including in those with hypovitaminosis B12^{351,353} and hyperhomocysteinemia.³⁵⁷

Treatment type

- **Compounds**

- Glutathionylcobalamin
 - used by neurons under oxidative stress conditions¹⁴⁶
 - not commercially available^{146,290}
- Cyanocobalamin
 - not used by neurons under oxidative stress conditions^{146,290}
 - commercially available
- Hydroxocobalamin
 - not used by neurons under oxidative stress conditions^{146,290}
 - commercially available
- Mecobalamin
 - not used by neurons under oxidative stress conditions^{146,290}
 - commercially available

- **Routes of administration**

- Cyanocobalamin
 - can be administered orally, sublingually, intranasally, intramuscularly, subcutaneously, or intravenously
 - The intravenous route is not advised unless the patient is in renal failure, since more than 98% of an intravenous dose may be lost in the urine.
- Methylcobalamin
 - Oral doses
 - do not increase serum or cerebral spinal fluid B12 levels⁴⁹⁰
 - do not improve cognitive function or activities of daily living in patients with Alzheimer's disease³⁶¹
 - Intramuscular or intravenous doses are required
 - Intravenous doses have been shown to improve cognition, communication, emotions, and memory in patients with Alzheimer's disease.⁴⁷⁵

- **Whether or not methyl donors or antioxidants are administered**

- N-acetylcysteine, a cell-permeate glutathione precursor,⁴⁸³ when combined with B12, improves cognition in patients with HHcy.^{474,488}
- S-adenosylmethionine may be a useful adjunct for treatment of depression,^{477–479,485,487} associated with Parkinson's disease,⁴⁸¹ human immunodeficiency virus infection,⁴⁹⁴ and fibromyalgia.⁴⁹⁵
- Betaine lowers systemic Hcy^{476,482,491,492} and may delay illness progression in Alzheimer's disease.⁴⁸⁶
 - Homocysteine methylation to methionine occurs by one of two means
 - methionine synthetase converting methyltetrahydrofolate to tetrahydrofolate, with methyltetrahydrofolate as the methyl group donor – occurs in all cells
 - betaine homocysteine methyltransferase converting betaine to dimethylglycine, with betaine as the methyl group donor^{17,58,484} – confined to the liver and kidneys^{480,489,493} This may not matter in the treatment of dementia, as a leaky blood brain barrier may allow influx of systemic homocysteine.

contrary results or recommendations.^{27,41,62,188,340,398,401,506–508} Possible errors from this review and its conclusions are that Medline was the primary database, searches may not have captured all relevant studies and case reports, and it is presumed that there are unpublished vitamin B12 treatment responses. In order to attempt to minimize erroneous conclusions, results were crosschecked with Internet searches and basic and clinical science textbooks, other Medline searches were performed using various search terms, and communication took place with experts who care for demented patients. Owing to the latter, it is crucial that PA be ruled out in all demented patients. Also, the trials that have been reported to date may have been of insufficient size or duration to determine a beneficial effect.²³³

Biological data support the notion that ROS generation may lead to elevated Hcy, but this may be, at least in part, an adaptive mechanism to generate cysteine, which is used in the biosynthesis of glutathione,^{120,509} an antioxidant that protects cells from oxidative stress. Biochemical and epidemiological evidence is convincing that HHcy, with or without hypovitaminosis B12, is a risk factor for dementia. Evidence is less convincing that hypovitaminosis B12 is a risk factor for dementia in the absence of HHcy. B12 deficiency manifestations are variable and include abnormal psychiatric, neurological, gastrointestinal, and hematological findings. Radiological images of nondemented and demented individuals with HHcy frequently demonstrate leukoaraiosis, potentially related to BBB dysfunction. Thus, the finding of leukoaraiosis on CAT or MRI scan is an indication for checking plasma Hcy and serum B12 levels.

Although historically neuropathy related to PA was described according to a classical disease progression, patient samples now show that symptoms and signs secondary to the neuropathy can be variable.^{180,188} Nonetheless, dementia secondary to PA, occurring separately from other manifestations of SCD or peripheral neuropathy, and in the absence of macrocytosis and anemia, may still be considered sufficiently rare to be the basis of case reports.^{190,199,434}

On the one hand, literature suggests assessing serum B12 and treatment of B12 deficiency is useful early in the course of cognitive dysfunction, for instance, MCI^{199,350,469} and mild to moderate dementia of less than two years duration.^{24,180,346,356,358,359} If low serum B12 is determined, it may be worthwhile to check for antiparietal cell and anti-intrinsic factor antibodies and obtain testing for *H. pylori*, for management and prognostic purposes. On the other hand, literature suggests assessing serum B12 and treatment of B12 deficiency may not be useful late in the

course of cognitive dysfunction, for instance, moderately severe to severe dementia, if symptoms and signs of SCD and peripheral neuropathy, hematological manifestations of PA, and other factors listed on Figure 4 are absent. However, if one has not inquired about neurological symptoms, examined the mouth and tongue, performed a neurological examination, and obtained a CBC on a moderately-severe to severely demented patient, then it could be a ‘medical-legal pitfall’ not to check the serum B12 level.⁶² The suggestions for vitamin B12 workup and treatment in patients with suspected MCI or dementia are evidence-based. However, since the rate of dementia progression is poorly measured and variable and there are no specific criteria for vitamin B12 testing, the suggestions are not intended to replace care based upon clinical decisions. The serum B12 level remains the standard initial test.¹⁸¹ DAT is a compatible diagnosis when B12 deficiency is found, unless it is caused by PA. Cyanocobalamin PO is favorable over IM, in terms of both therapeutic efficacy and patient preference, unless there is concern regarding PO adherence.

Since the original observations by Biermer in 1872, to the more recent epidemic neuropathy in Cuba, it is evident that adequate folate and vitamin B12 function is required for physical and mental health. Issues surrounding folate, vitamin B12, Hcy, and MMA as they relate to health, especially microvascular disease and cognitive dysfunction, are high interest among researchers and clinicians. Puzzling questions remain. Since B12 deficiency can lead to depression, mania, and psychoses, should clinicians routinely check serum B12 levels in patients with such findings? Among those individuals who are B12 deficient, what are the prevalences of antiparietal, anti-intrinsic factor, and anti-*H. pylori* antibodies? Is HHcy a risk factor for other types of dementia, such as DBT, dementia due to PD, or dementia with Lewy bodies?^{193,510,511} Since HHcy has been prospectively shown to be an ‘independent’ risk factor for dementia, then why are Hcy-lowering therapies ineffective, or at best modestly effective, at preventing or treating dementia? Answers to questions such as these may one day unleash knowledge necessary for more effective treatment. The notions of age-related, and perhaps disease-related, degradation of the BBB and oxidative chemical reactions, generating ROS and elevating Hcy, coupled with immune activation, both without and within the brain, remain novel areas for further work.¹²⁰

Acknowledgments

The author wishes to thank the reviewers of this manuscript, for their thoughtful work and kind suggestions, which were

highly valuable in the final writing of this publication. The author reports no conflicts of interest in this work.

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