Homocysteine in relation to cognitive performance in pathological and non-pathological conditions

Charlotte E. Teunissen1,*, Martin P. J. van Boxtel2, Jellemer Jolles2, Jan de Vente2, Fred Vreeling3, Frans Verhey2, Chris H. Polman4, Christine D. Dijkstra1 and Henk J. Blom5

1 Department of Molecular Cell Biology and Immunology, Amsterdam, and VUmc Medical Center Amsterdam, Amsterdam, The Netherlands
2 Department of Psychiatry and Neuropsychology, Maastricht University, Maastricht, The Netherlands
3 Department of Neurology, Academic Medical Center Maastricht, Maastricht, The Netherlands
4 Department of Neurology, VUmc Medical Center Amsterdam, Amsterdam, The Netherlands
5 Laboratory of Pediatrics and Neurology, University Medical Center Nijmegen, Nijmegen, The Netherlands

Abstract
Elevated serum homocysteine has been associated with increased risk of Alzheimer’s disease. Furthermore, elevated homocysteine levels are related to cognitive dysfunction in the elderly. The aim of the present study was to explore the disease specificity of the relation between serum total homocysteine levels and cognitive function. For this, we summarize data from several studies on homocysteine levels in both normal and pathological conditions performed in our laboratories and evaluate possible mechanisms of effects of elevated homocysteine levels in the central nervous system. Total homocysteine levels were measured in serum of: 1) healthy aging individuals; 2) patients with Alzheimer’s and Parkinson’s disease and patients with other cognitive disorders; and 3) patients with multiple sclerosis. Increased serum homocysteine concentration was related to worse cognitive performance over a 6-year period in the normal aging population (r = -0.36 to -0.14, p < 0.01 for the Word learning tests; r = 0.76, p < 0.05 for the Stroop Colored Word test). Homocysteine was only increased in patients with Parkinson’s disease on L-Dopa therapy (18.9 vs. 16.5 μmol/L in healthy controls), and not in dementia patients. Homocysteine was elevated in patients with progressive multiple sclerosis (15.0 μmol/L, n = 39, compared to 12.0 μmol/L in 45 controls) and correlated to both cognitive and motor function (r = -0.33 and -0.33, p < 0.05, respectively). The relationship between homocysteine and cognitive function in non-pathological and pathological situations indicates that changes in its levels may play a role in cognitive functioning in a broad spectrum of conditions.

Keywords: aging; cognition; dementia; homocysteine; multiple sclerosis.

Introduction
Elevated total serum homocysteine has been related to cognitive functioning. Increased plasma homocysteine levels in patients with Alzheimer’s disease (AD) were initially reported by Regland et al. in 1990 (1) and in histologically confirmed cases by Clarke et al. in 1998 (2), and the epidemiological literature suggesting a relation between homocysteine and AD has grown rapidly since. Recently, elevated plasma homocysteine concentration has been shown to elevate the risk of AD (3). A relation between elevated plasma homocysteine concentration and decreased cognitive performance has also been observed in the normal aging population (4–7).

Levels of other molecules involved in the transmethylation pathway, such as vitamins B6, B12 and folate, have also been investigated in relation to cognition. A cross-sectional correlation between vitamin B12 and folate and cognitive performance has also been suggested, though this is less clear than for homocysteine (8). The fact that homocysteine has been related to cognition in different conditions, pathological or during normal aging, raises the question as to whether this relation is disease-specific. The aim of the present study was to explore the disease specificity of the relation between serum total homocysteine levels and cognitive function. For this, we summarize the results on serum homocysteine levels from different studies performed in our laboratories: 1) a longitudinal follow-up study of normal aging individuals; 2) a study in patients with AD and Parkinson’s disease (PD) and patients with other cognitive disorders; and 3) a study including patients with multiple sclerosis (MS). In addition, possible mechanisms of the effects of elevated homocysteine levels on the central nervous system (CNS) are discussed.

Longitudinal follow-up study of normal aging individuals
Longitudinal studies investigating a possible risk-modulating role of elevated serum homocysteine on aspects of normal cognitive aging are still rather
scarce. At the start of our analyses, only two prospective studies had been performed correlating homocysteine concentrations to cognitive decline as measured by a decrease in Mini-Mental State Exam (MMSE) score in the normal aging population (5, 9). In the present study, we correlated the serum concentrations of homocysteine, vitamin B₁₂ and folate with the individual cognitive performance on domains of information processing speed, learning, memory and attention during a follow-up of 6 years. This study was published in detail previously (10).

Normal aging individuals were participants of the Maastricht Aging Study (MAAS), a larger research program investigating determinants of cognitive aging in the healthy population. Individuals over 50 years old were thoroughly tested every third year, and individuals below the age of 50 years every sixth year (11, 12). When we started serum analysis at the end of 1999, cognitive data over 6 years of follow-up were available from a random group of 144 individuals from the panel that had their baseline visit in 1993. A complete set of serum samples, in addition to the cognitive performance data, was available from 93 individuals at baseline and from 115 individuals at follow-up. A complete set of serum samples was available from 65 individuals at both time points. Incomplete sets of serum samples were due to technical problems. None of the subjects used B-vitamin supplementation. Cognitive function testing comprised the Word learning test for learning and memory: total recall of a series of 15 monosyllabic words presented in five consecutive trials (Word learning test, total words: WLTTOT) and recall after 20 min (Delayed Recall); a perceptual interference test (Stroop Color-Word Test); and the Letter-Digit coding test (LDCT) as a measure of information processing speed.

The results showed that serum homocysteine concentrations at baseline correlated negatively with performance on the WLTTOT and Delayed Recall test, adjusted for age, sex and education at baseline. However, these correlations were absent at follow-up. No correlation of the serum homocysteine concentrations and performance on the Stroop test or the LDCT was observed at any of the time points. Next, we investigated the relation between serum concentrations of homocysteine at baseline and cognitive performance over the whole follow-up period to investigate any eventual risk-modulating role of elevated homocysteine concentrations. This was carried out using multi-level repeated-measurement analysis. The results presented in Figure 1 show that the serum homocysteine concentrations at baseline correlated negatively with test scores on the WLTTOT and Delayed Recall test over the 6-year follow-up period (regression coefficients, −0.36, p < 0.01 and −0.14, p < 0.05, respectively). These results indicate prolonged relatively worse performance when homocysteine concentrations were high at baseline. The serum homocysteine concentrations at baseline also correlated positively with test scores on the Stroop test over 6 years of follow-up (regression coefficient, 0.76, p < 0.05). No results could be obtained for a risk-modulating role of elevated homocysteine on cognitive functioning, since there were no significant differences between persons in the course of cognitive function during the 6-year period of this study. This may be due to the relatively young age of this population combined with the relatively short follow-up period of 6 years.

So far, the few longitudinal follow-up studies performed on homocysteine levels in relation to cognitive decline in the elderly have shown variable results. Kalmijn et al. (5) showed no relation between homocysteine concentrations and change in the MMSE score over a period of 2.7 years, an even shorter follow-up period than in our investigations. Another follow-up study showed a correlation between homocysteine concentrations and decline in one point of the MMSE score (from 29 to 28) over 5 years. However, the power in this study was very small (n = 23) and the data were only corrected for gender and not for age (9). It may also be questioned as to whether the MMSE is sensitive for detecting differences in cognitive functioning in the normal aging population. A recent large study (n = 370) investigated the levels of homocysteine and the vitamins folate, B₆ and B₁₂ in relation to cognitive decline after 7 years in older (70–79 years) adults who were in the top third of cognitive and physical functioning. Regression models for cognitive decline as dependent variables and the serum markers as independent variables showed an
independent effect on cognitive decline only for folate. Homocysteine levels at baseline were related to cognitive decline, but this effect was mediated by folate levels (13). It has been reported that 19–50% of people with mild cognitive impairment may develop AD (14), but it is not yet clear whether cognitive change during non-pathological aging proceeds to the development of clinical AD.

Comparison of different patient groups with “classical” neurodegenerative diseases

Elevated plasma homocysteine levels have been associated with AD. For example, increased homocysteine concentrations were shown to be a risk factor for AD after 8 years of follow-up (n = 499) (3). This result was largely mediated by low folate levels. In another study (n = 679) no significant risk-elevating effect of high homocysteine on the development of AD was observed (15). Serum homocysteine levels have been shown to be increased in AD patients in some studies (16), but not in all (17). Serum homocysteine levels are increased in PD patients on L-Dopa therapy (18, 19). However, no differences in serum or cerebrospinal fluid (CSF) levels of homocysteine were observed in patients with Creutzfeldt-Jakob disease compared to healthy controls (n = 13 per group) (20).

It is unclear whether homocysteine is related to cognition in a broad range of neurodegenerative diseases or whether it is specific for disorders primarily affecting cognition. Therefore, we compared the serum homocysteine levels in several neurodegenerative diseases.

We investigated serum total homocysteine levels in AD patients (n = 34, mean age 73, range 53–95 years), patients with PD (n = 46, mean 70, range 42–89 years), patients with other cognitive diseases (OCD; n = 47, mean 70, range 52–85 years) and healthy individuals (n = 61, mean 68, range 55–86 years) (21). No significant differences in mean age were observed between the groups. The OCD patients (n = 47) were diagnosed as having cognitive disturbance (DSM code 294.9) (n = 14), mild cognitive impairment (n = 6), vascular dementia (n = 7), non-specific dementia (n = 5), fronto-temporal dementia (n = 4), depression-induced dementia (n = 4), primary aphasia (n = 4), brain tumor (n = 1) and unknown (n = 2).

The results showed that increased homocysteine levels were only observed in the patients with PD (18.9 vs. 16.5 μmol/L in healthy controls). Within this group, the high homocysteine levels were observed in patients on L-Dopa therapy. Adjustment of the data for age as a possible confounder did not reveal a difference between the different groups. A weak negative correlation was observed between homocysteine and MMSE scored within 0.5 months (SD = 1.4) (Spearman’s r = 0.18, p < 0.05, n = 134). This association was not found in the AD group separately (r = 0.023, p > 0.05, n = 21). It is possible that population characteristics caused the lack of results in our study compared to the elevated homocysteine levels in AD patients in other studies. Alternatively, it is likely that the relatively small increases in homocysteine levels can only be observed in studies with large group sizes, which also limits the significance for individuals.

Serum homocysteine in a “new” neurodegenerative disease: multiple sclerosis

MS is known as an inflammatory demyelinating disease. MS can be classified into three clinical subtypes based on the disease course: 1) the relapsing-remitting (RR) subtype, with a prevalence of 45%; 2) the secondary progressive (SP) subtype, with a prevalence of 35%, which usually follows the RR phase; and 3) the primary progressive (PP) subtype, with a prevalence of 20%. The importance of axonal degeneration in MS has become clear in recent years, which may mean that MS can be classified as a “new” neurodegenerative disease. Axonal loss has now been implicated as an important feature of MS, since it occurs early in the disease course and is the likely correlate of irreversible neurological and cognitive decline in MS. Brain parenchymal and ventricular fraction, surrogate markers for axonal loss, were shown to correlate to cognitive decline in MS, adjusted for age, premorbid intelligence and depression (22). Cortical volume, indicating gray-matter lesions, was also negatively correlated to several tests of memory and attention in cognitively impaired MS patients (23). Cognitive disturbances are prevalent among 40–60% of MS patients and the numbers increase during progression of the disease (24–26). To date, no serum biomarkers have been found to correlate with axonal loss in MS (27).

In previous studies, elevated homocysteine levels were observed in MS patients with vitamin B deficiency (28–30). In a recent study, elevated homocysteine levels were reported in a total group of 72 MS patients compared to 23 controls (31). In a pilot study, we set out to investigate serum homocysteine in the different clinical subtypes of MS and to relate these levels to the scores of clinical functioning. Sera were obtained from 19 RR MS patients (mean age ± SD, 42.7 ± 10.4 years), 19 SP MS patients (45.5 ± 6.9 years), 20 PP MS patients (51.6 ± 9.2 years) and 45 controls (43.7 ± 12.4 years).

In the overall group of MS patients, increased total homocysteine levels (mean ± SD) were observed compared to controls (MS, 13.3 ± 5.4 μmol/L, n = 58; controls, 12.3 ± 3.2 μmol/L, n = 45; p < 0.05). These increases were mainly found in patients with a progressive form of the disease (SP, 15.0 ± 5.9 μmol/L, n = 19; PP, 15.3 ± 4.7 μmol/L, n = 20; p < 0.05 for each subtype compared to controls). None of the RR MS patients were in relapse, and therefore it is still possible that homocysteine levels are related to acute clinical activity. Next, a positive correlation with the expanded disability scale score (EDSS) was observed (r = 0.413, p < 0.001, adjusted for age) and a negative correlation with the MS functional composite test (MSFC, r = −0.330, p < 0.03, adjusted for age), indicating worse functioning in patients with increased
homocysteine levels. To elucidate whether a relationship was present with aspects of cognitive functioning, the homocysteine levels were related to subtests of the MSFC. Similar correlations were present in the subtests for both cognition and locomotion ($r = -0.33$ and $-0.33$, $p < 0.016$, adjusted for age). These results indicate a relation between homocysteine and cognition, and also locomotion, in MS. The results need to be interpreted with some caution, since in the correlation with cognitive functioning we did not correct for educational levels or formal intelligence level, which has recently been shown to influence the cognitive abilities of MS patients (26). The relation between serum homocysteine and locomotion is supported by a relation between serum homocysteine levels and decline in physical function in the elderly (32). The presence of elevated homocysteine in progressive phases argues against a causal relation with neurological decline in MS.

**Overall conclusion**

The results of our studies and those of others indicate that homocysteine can be related to various aspects of cognitive and neurological functioning. This was observed in the normal aging population, in MS patients and in the total group of neurodegenerative patients. No clear increase was observed in the AD or PD groups separately. These results may suggest that homocysteine is a state-marker of cognitive functioning and is likely independent of specific neurological conditions. This is also supported by results from different published studies, as indicated in the Introduction. Nevertheless, some diseases may be an exception, such as Creutzfeldt-Jakob disease (20), and are therefore very interesting. This disease independence could implicate a common mechanism: all conditions may share a common neurodegenerative mechanism leading to an imbalance in transmethylation pathways or a common defect in transmethylation pathways that causes cognitive, and possibly physical, decline. The main question that then arises is the possible mechanism, which is hard to identify by correlational studies. Not much is known about the relation between blood homocysteine levels and ongoing processes in the brain. So far, almost all studies have investigated total homocysteine levels in serum/plasma. CSF might be a better reflection of CNS processes, since it is closest to the brain parenchyma. The few studies correlating plasma to CSF levels gave inconclusive results (31, 33).

**Possible mechanisms in the CNS**

The transmethylation pathway is active in every CNS cell, although there are regional differences (34).
effects of homocysteine on cellular functions could be exerted both intracellularly and extracellularly (Figure 2). Furthermore, effects could be due to an imbalance in the CNS parenchyma or via effects on the vasculature, such as occurs in atherosclerosis and stroke (36). For example, homocysteine has been related to a risk of silent brain infarcts or atherosclerotic white-matter lesions in the elderly (37).

Oxidative stress is a likely mechanism associated with elevated homocysteine levels. In Alzheimer patients, elevated homocysteine levels were correlated to plasma levels of lipid peroxidation products (33). Oxidative stress could be an effect of elevated homocysteine levels, for example, due to autoxidation of its sulphydryl group (38). Indeed, protective effects of antioxidants have been reported on cognitive dysfunction and elevated CNS levels of oxidation products induced by homocysteine in rats (39, 40). No studies are available yet that examine the effects of antioxidant supplementation on cognitive function in relation to homocysteine levels in humans. Elevated homocysteine can also lead to oxidative stress via excitotoxicity. Homocysteine can induce excitotoxicity by agonising the effects of glutamate (41, 42) or indirectly via an effect on the Na⁺/K⁺ pump, as has been observed after supplementation of homocysteine in young rats (43). This pump is important for maintaining the membrane potential and functioning of several receptors. Excitotoxicity leads to increased intracellular Ca²⁺ influx, which affects many cellular functions and ultimately leads to severe oxidative stress and cell death. It is also possible that elevated homocysteine levels are induced by oxidative stress.

For example, supplementation with the antioxidant acetylcysteine reduced plasma homocysteine levels and improved endothelial function in a placebo-controlled crossover study in patients with end-stage renal failure (44). In addition, mice with a mutation in the superoxide dismutase enzyme that led to plasma levels of lipid peroxidation products induced by homocysteine in rats (39, 40). No studies are available yet that examine the effects of antioxidant supplementation on cognitive function in relation to homocysteine levels in humans. Elevated homocysteine can also lead to oxidative stress via excitotoxicity. Homocysteine can induce excitotoxicity by agonising the effects of glutamate (41, 42) or indirectly via an effect on the Na⁺/K⁺ pump, as has been observed after supplementation of homocysteine in young rats (43). This pump is important for maintaining the membrane potential and functioning of several receptors. Excitotoxicity leads to increased intracellular Ca²⁺ influx, which affects many cellular functions and ultimately leads to severe oxidative stress and cell death. It is also possible that elevated homocysteine levels are induced by oxidative stress.

The effects of homocysteine on the vasculature likely involve nitric oxide (NO). NO and homocysteine can form nitrosohomocysteine, thereby modulating their bioavailability and biological effects (46–48). In addition, nitrosohomocysteine inhibits apoptosis induced by homocysteine on endothelial cells (49). In cortical neurons, relative lower toxicity was observed for nitrosohomocysteine compared to homocysteine (50). In contrast, another study showed toxicity induced by nitrosohomocysteine and not by homocysteine in cultured cortical neurons, which was attributed to the formation of peroxynitrite by NO (51). The differences could be explained by the finding in the latter study (51) that the ratio of homocysteine to the nitrosylated compound determined the outcome. NO is known to have a dual role in the CNS, which may also account for the differential effects (52, 53). It is toxic under conditions of oxidative stress due to the formation of the radical peroxynitrite, while there are indications that it can be protective under other conditions. For example, knockout of nNOS led to delayed regeneration of peripheral nerves (54). There are other possible mechanisms by which homocysteine and NO homeostasis are related, as discussed elsewhere in this issue.

Other mechanisms of homocysteine-induced effects are the inhibition of DNA repair mechanisms (55) or the decreased availability of methionine, which is essential for methyl transfer via S-adenosylmethionine, one of the mechanisms involved in the synthesis and degradation of neurotransmitters (56). Hyperhomocysteinemia can increase S-adenosylhomocysteine, which inhibits methyltransferases, the activity of which was shown to be decreased in AD brains (35).

In conclusion, there are several indications of a relation between elevated homocysteine levels and neurological functioning. So far, the relation between homocysteine and cognition is not specific for a single pathological or non-pathological state. The most important question now is to resolve the biological implications of this relation to allow a rational approach towards possible interventions.

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References


