The Plasma Homocysteine Concentration Is Better Than That of Serum Methylmalonic Acid as a Marker for Sociopsychological Performance in a Psychogeriatric Population

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Background: Cobalamin/folate deficiency in elderly subjects may lead to psychiatric symptoms, but more often it increases the severity of various organic and nonorganic mental diseases. A major clinical problem, however, is the uncertainty and controversy concerning biochemical markers of cobalamin/folate deficiency to be used in the diagnostic evaluation of suspected cobalamin/folate deficiency.

Methods: We measured plasma homocysteine (tHcy), blood folate, serum methylmalonic acid, and serum cobalamin in 80 psychogeriatric patients (age, 77.3 ± 8.6 years) and 50 controls (age, 76.1 ± 8.0 years). We assessed associations of these tests with measures of cognitive and behavior performance by use of regression analyses.

Results: Plasma tHcy was increased in 45% of the psychogeriatric population (mean, 20.5 ± 9.2 μmol/L vs 15.3 ± 4.7 μmol/L for controls; P < 0.01). Plasma tHcy correlated with severity of dementia (r = 0.36; P < 0.01), the Katz ADL index (r = 0.29; P < 0.05), the Berger scale (r = 0.29; P < 0.05), and the score of symptoms (r = 0.39; P < 0.001) in the psychogeriatric population. Similarly, blood folate was significantly correlated with these measures, but the concentrations of serum cobalamin and methylmalonic acid were not. In a stepwise multiple regression analysis including the biochemical markers, tHcy was the only significant predictor of the severity of dementia (r² = 0.11; P < 0.01) and the score of symptoms (r² = 0.16; P < 0.001).

Conclusion: Plasma tHcy is the best marker of those measured to investigate suspected tissue deficiency of cobalamin/folate.

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The investigation of mental diseases in elderly subjects reveals a large variety of organic and nonorganic etiologies, very often working in combination. A variety of metabolic and nutritional disorders may cause or contribute to a chronic brain syndrome. Thus, cobalamin/folate deficiency in elderly subjects may lead to psychiatric symptoms, but more often, it increases the severity of various organic and nonorganic mental diseases. It is also possible that cobalamin/folate deficiency might be a consequence of poor dietary intake in dementia. The metabolism of folate and cobalamin is intimately connected, and this is reflected in the fact that a deficiency of either vitamin may eventually lead to morphologically indistinguishable megaloblastic anemia in humans and may also produce several neurological and psychiatric sequelae (1–5). Cobalamin/folate deficiency has been related to cognitive impairment and dementia in several studies (1–8). The neuropsychiatric complications of folate and cobalamin deficiency are much less clearly understood than the hematologic manifestations. A major clinical problem, however, is the uncertainty and controversy concerning which biochemical markers of cobalamin/folate deficiency should be used in the diagnostic process (9). Most of the studies of the relationship between mental disorders in the elderly and deficiencies of cobalamin and folate have used methods that determine the blood concentrations of these vitamins, which might not reflect the vitamin status in the tissues (1–8).

Recently two new markers, plasma total homocysteine (tHcy) and methylmalonic acid, have attracted growing interest because they are considered to reflect the status of cobalamin and folate in the tissues. Homocysteine is formed in the transmethylation process (10) when S-
adenosylmethionine is converted to S-adenosylhomocysteine, which in turn is converted to homocysteine. Homocysteine can be remethylated to methionine mainly by the folate- and cobalamin-dependent enzyme methionine synthase (10). Plasma concentrations of tHcy have been shown to be increased in patients with cobalamin and/or folate deficiency (11, 12). Cobalamin is also an essential cofactor in the enzymatic conversion of methylmalonyl-CoA to succinyl-CoA by the enzyme L-methylmalonyl-CoA mutase (13); cobalamin deficiency therefore leads to an increased serum concentration of methylmalonic acid (12, 14). In a large study of 406 patients (12) with clinically defined cobalamin deficiency, almost every patient had increased methylmalonic acid and/or homocysteine. It was concluded that methylmalonic acid and tHcy concentrations within reference values rule out clinically significant cobalamin deficiency. In the same study, it was reported that serum tHcy was increased in ~90% of those subjects with folate deficiency who had a clinical response to folate supplementation.

There is no consensus about which biochemical marker of cobalamin/folate deficiency to be used in the diagnostic evaluation of vitamin deficiencies and which of these best reflects the functional availability of cobalamin and folate. It is therefore of importance to compare the association of plasma tHcy, blood folate, serum methylmalonic acid, and serum cobalamin with functional indicators of the central nervous system (such as different forms of cognitive and behavior performance) to evaluate the best marker to determine in patients suspected of tissue deficiency of cobalamin/folate.

Materials and Methods

STUDY POPULATIONS
The present study population consisted of 80 patients (44 women and 36 men) with symptoms of organic brain disease and referred to the Psychogeriatric Department at Lund University Hospital for diagnostic examination and treatment. Patients on any kind of ongoing vitamin supplementation were excluded from the study. Similarly, patients with mainly nonorganic mental diseases and patients with severe somatic diseases in terminal stages were excluded from the study. The mean age of these patients was 77.3 ± 8.6 years. The majority of cases were living in their own homes, alone or with relatives. Organic dementia was diagnosed in all of the patients (21 patients with dementia of Alzheimer type, 4 patients with primary degenerative fronto-temporal dementia of non-Alzheimer type, 35 patients with vascular dementia, 3 patients with an unspecified dementia condition, 5 patients with other specified types of dementia, and 12 patients with mixed vascular and Alzheimer-type dementia). The clinical diagnosis was based on psychiatric, neurological, somatic, and laboratory investigations, psychometric testing, measurements of regional cerebral blood flow, encephalography, and computed tomography scans or magnetic resonance imaging as described previously (15–17).

No patients had macrocytotic anemia. The study was approved by the ethics committee of the University of Lund. Informed consent to participate was given by all subjects (or relatives if the patients were unable to communicate).

Fifty apparently healthy subjects (20 women and 30 men; mean age, 76.1 ± 8.0 years), randomly invited on the basis of a local population register as described previously (18), served as a control group for the biochemical markers. They had no history of actual or previous institutional care for renal, hepatic, or mental disease and were taking no vitamin supplements. They were living in their own homes.

ASSAYS
Blood samples for homocysteine determination were collected in evacuated tubes containing EDTA at approximately 0800 after an overnight fast and centrifuged within 15 min at 3000g for 5 min. The plasma was stored at −20 °C until analysis. Plasma tHcy was measured as described previously (19) on an HPLC after reduction of disulfide bonds with dithiothreitol and deproteinization with sulfosalicylic acid. The method has a CV of 4%, calculated at 15, μmol/L. The assay of methylmalonic acid was performed as described previously (20) on a Finnegan magnum ion trap gas chromatography–mass spectrometry system. The CV was calculated as 4% at 0.5 μmol/L. The upper reference limit (95th percentile) for plasma homocysteine in an elderly population is 19.9 μmol/L (15), and that for plasma methylmalonic acid is 0.41 μmol/L (17).

Serum cobalamin and blood folate were determined by RIA, using purified intrinsic factor and purified folate-binding protein at the Department of Clinical Chemistry, Lund, University Hospital (Vitamin B₁₂/Folate Dual RIA kit; Amersham). The reference interval for blood folate was 125–500 nmol/L. In the present and earlier studies (15–17), we used 150 pmol/L as the lower reference limit for serum cobalamin because this value is used in most studies in this research field. Serum creatinine (upper reference limit, 120 μmol/L), blood hemoglobin, and the mean cell volume of erythrocytes were assayed with routine methods at the Department of Clinical Chemistry.

The severity of dementia was classified according to DSM-III-R as mild, moderate, or severe (21). The Katz ADL index (22) was used to assess the physical dependency of the patients, and the Berger scale (23) was used to measure the social dependency of the patients and their need of caring support. Both scales are hierarchically constructed according to the common pattern of dementia progression. The dementia progression is described in six steps in both methods. The records of the patients were evaluated, and symptoms of cognitive deterioration and other symptoms indicating an organic brain disease were assessed, classified, and scored independently by two experienced nurse-specialists trained in dementia care. The occurrence and the severity of symptoms were eval-
A comprehensive analysis was performed. Patients with different severities of dementia, Katz ADL index, Berger scale, and scores of symptoms were summarized with a maximum score of 23.

**Statistics**

The results are presented as mean ± SD. The following two-tailed tests at the 5% level of significance were used to evaluate the study: The Mann–Whitney U-test was used in the case of two independent samples, and the Spearman rank correlation coefficient test was used to test for monovariate relationships between different variables. The $^{2}$ test was used for comparisons among groups of patients with different severities of dementia, Katz ADL indices, Berger scales, and scores of symptoms. In the case of more than two variables, stepwise multiple regression analysis was performed.

**Results**

In the psychogeriatric population, the blood folate concentration was decreased and the plasma tHcy concentration was increased, whereas the concentrations of methylmalonic acid and serum cobalamin were not changed compared with the healthy subjects (Table 1). Seven patients in the psychogeriatric population could be classified as cobalamin-deficient because they had appropriate changes of all three markers for cobalamin deficiency (serum cobalamin <150 pmol/L, plasma tHcy >19.9 μmol/L, and plasma methylmalonic acid >0.41 μmol/L). The other 73 patients exhibited increased plasma tHcy and decreased blood folate compared with healthy subjects.

The plasma tHcy concentration was increased in 36 of the 80 patients. Twelve of these patients also had increased serum methylmalonic acid and/or decreased serum cobalamin (Table 2). Two additional patients had increased serum creatinine. In two patients blood folate was decreased. Twenty-two patients had only increased plasma tHcy.

The patients were divided into three groups according to the severity of the dementia (Table 1), and as expected, the Katz ADL index, the Berger scale, and the symptom score were significantly increased in patients with moderate and severe of dementia compared with the patients with mild dementia. Similarly, plasma tHcy was increased and blood folate was decreased in the group with severe dementia compared with the group with mild dementia, whereas the serum cobalamin and methylmalonic acid concentrations were similar in all groups. Exclusion of the seven cobalamin-deficient patients did not change this finding.

Correlation studies were also performed in all patients ($n = 80$). Plasma tHcy and plasma methylmalonic acid

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**Table 1. Katz ADL index, Berger scale, score of symptoms, and concentrations of plasma homocysteine, blood folate, serum methylmalonic acid, serum cobalamin, and serum creatinine in different groups of dementia.**

<table>
<thead>
<tr>
<th>Dementia</th>
<th>n</th>
<th>Patients</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>76.1 ± 8.0</td>
<td>77.3 ± 8.6</td>
<td>76.4 ± 9.3</td>
<td>75.7 ± 8.5</td>
<td>80.6 ± 7.4</td>
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<tr>
<td>Katz ADL index</td>
<td>3.1 ± 1.8</td>
<td>3.0 ± 1.3</td>
<td>5.2 ± 1.0</td>
<td>4.6 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Berger scale</td>
<td>3.0 ± 1.4</td>
<td>3.1 ± 0.9</td>
<td>4.6 ± 0.6</td>
<td>2.9 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Score of symptoms</td>
<td>8.9 ± 4.3</td>
<td>8.5 ± 2.6</td>
<td>14.3 ± 3.2</td>
<td>12.1 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>P-Hcy, μmol/L</td>
<td>15.3 ± 4.7</td>
<td>20.5 ± 9.2</td>
<td>16.7 ± 5.9</td>
<td>19.6 ± 8.9</td>
<td>25.2 ± 10.5</td>
</tr>
<tr>
<td>B-Fol, nmol/L</td>
<td>355 ± 179</td>
<td>282 ± 96</td>
<td>316 ± 78</td>
<td>310 ± 156</td>
<td>245 ± 82</td>
</tr>
<tr>
<td>S-MMA, μmol/L</td>
<td>0.27 ± 0.13</td>
<td>0.28 ± 0.18</td>
<td>0.25 ± 0.14</td>
<td>0.28 ± 0.2</td>
<td>0.29 ± 0.18</td>
</tr>
<tr>
<td>S-Cob, pmol/L</td>
<td>258 ± 126</td>
<td>258 ± 121</td>
<td>266 ± 139</td>
<td>268 ± 121</td>
<td>234 ± 105</td>
</tr>
<tr>
<td>S-Creat, μmol/L</td>
<td>91 ± 27</td>
<td>79 ± 21</td>
<td>74 ± 21</td>
<td>77 ± 17</td>
<td>81 ± 24</td>
</tr>
</tbody>
</table>

**Table 2. Distribution of the different determinants for plasma homocysteine concentration in 36 patients with increased plasma homocysteine (>19.9 μmol/L).**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>S-Cob* &lt;150 pmol/L</th>
<th>S-MMA* &gt;0.41 μmol/L</th>
<th>B-Fol &gt;125 nmol/L</th>
<th>S-Creat &gt;120 μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
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<td>0</td>
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<tr>
<td>4</td>
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<td>2</td>
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<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>36 (total)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a S-Cob, serum cobalamin; S-MMA, serum methylmalonic acid; B-Fol, blood folate; S-Creat, serum creatinine.
correlated with each other ($\rho = 0.40$; $P < 0.01$). Plasma tHcy also correlated with serum creatinine ($r = 0.37$; $P < 0.01$), age ($\rho = 0.38$; $P < 0.01$), and blood folate ($\rho = -0.44$; $P < 0.01$), and slightly with serum cobalamin ($\rho = -0.25$; $P < 0.05$). The plasma methylmalonic acid concentration correlated with serum cobalamin ($\rho = -0.57$; $P < 0.01$) and serum creatinine ($\rho = 0.28$; $P < 0.05$), but not with blood folate. There was also a significant correlation between serum cobalamin and blood folate ($\rho = 0.31$; $P < 0.01$) in this psychogeriatric population.

The severity of dementia, the Katz ADL index, the Berger scale, and the symptom score correlated with the plasma tHcy and blood folate concentrations in all patients ($n = 80$), but not with the serum cobalamin and methylmalonic acid concentrations (Table 3). Exclusion of the seven cobalamin-deficient patients did not change these results. A stepwise multiple regression analysis that included the biochemical variables showed that only the tHcy concentration predicted the severity of dementia ($r^2 = 0.11$; $P < 0.01$) and the score of symptoms ($r^2 = 0.16$; $P < 0.001$). Furthermore, the tHcy concentration was significantly (Spearman rank correlation test) correlated with symptoms reflecting cognitive functions, such as orientation ($P < 0.05$), remote memory disturbances ($P < 0.001$), learning ability ($P < 0.05$), and fluctuation of symptoms ($P < 0.001$), whereas symptoms such as aggression, depression, hallucinations, and delusions did not correlate with the tHcy concentration. The blood folate concentration also correlated significantly ($P < 0.05$) with symptoms reflecting cognitive functions, whereas there was no correlation between the methylmalonic acid concentration and any of the symptoms.

### Table 3. Correlations between concentrations of plasma homocysteine, blood folate, serum methylmalonic acid, and serum cobalamin and sociopsychological performance measured as severity of dementia, Katz ADL index, Berger scale, and score of symptoms.

<table>
<thead>
<tr>
<th></th>
<th>Severity of dementia</th>
<th>Katz ADL index</th>
<th>Berger scale</th>
<th>Score of symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma homocysteine</td>
<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.39&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blood folate</td>
<td>−0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−0.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum methylmalonic acid</td>
<td>0.11</td>
<td>0.08</td>
<td>0.06</td>
<td>0.11</td>
</tr>
<tr>
<td>Serum cobalamin</td>
<td>−0.08</td>
<td>0.03</td>
<td>−0.03</td>
<td>−0.09</td>
</tr>
</tbody>
</table>

<sup>a</sup> $P < 0.01$.

<sup>b</sup> $P < 0.05$.

<sup>c</sup> $P < 0.001$.

has been reported to be increased in depression (25) and in dementia of Alzheimer type (9, 26, 27).

Our main finding in the present study was the strong association between plasma tHcy/blood folate concentrations and sociopsychological performance (measured as severity of dementia, Katz ADL index, the Berger scale, and symptom score) in patients with dementia, whereas there was no association between serum methylmalonic acid or cobalamin concentrations and these variables. The scales used for measuring sociopsychological performance were the Katz ADL index, the Berger scale, and the occurrence of certain neuropsychiatric symptoms. The Katz ADL index is a hierarchical index that assumes that the loss of functions follows a predetermined order. The index considers performance of general hygiene, dressing, visits to the toilet, mobility, continence, and nutrition. The Berger scale is used to measure the social dependency and need of caring support. This scale is also hierarchically constructed, and the progress of the dementia from slight forgetfulness to bedridden, mutistic patient is described in six steps. The occurrence and severity of symptoms indicative of cognitive deterioration and behavioral disturbances or organic brain disease were evaluated and scored with a maximum score of 23. The findings of an association of tHcy concentration and sociopsychological performance are in agreement with a previous study of apparently healthy 80-year-old subjects in whom we observed a relationship between sociopsychological performance and plasma tHcy (28). There was a strong association between five indicators of well-being and lower plasma tHcy, whereas plasma methylmalonic acid was only weakly associated with one of these indicators (28 29). Recently, Lehmann et al. (30) observed, in accordance with our findings in the present study, that the plasma tHcy concentration was frequently increased (~40%) in a psychogeriatric population and correlated inversely with cognitive performance, whereas serum methylmalonic acid was not. McCaddon et al. (26) reported a significant relationship between cognitive function and plasma tHcy in Alzheimer disease. The plasma tHcy concentration has also been reported to relate to cognitive performance, measured as spatial copying performance, in healthy elderly subjects (31). Recently, it was reported (32) that in a random sample of 702 community-based subjects, ages 55 years and over, there was no relationship between the plasma tHcy concentration and cognitive impairment. However, compared with the studies described above, these subjects were younger and the cognitive impairment was measured only with the Mini-Mental State Examination.

A tissue deficiency of cobalamin or a combination of cobalamin/folate deficiency likely exists in the 12 patients with increased concentrations of both plasma tHcy and serum methylmalonic acid and/or decreased serum cobalamin or blood folate concentrations. Renal failure might explain increased concentrations of plasma tHcy in two other patients. Thus, as in the previous study (24),
there were many (n = 22) patients with increased plasma tHcy, which possibly can be attributed to a tissue deficiency of folate and/or cobalamin (24). It is not possible to exclude tissue deficiency of cobalamin in these 22 patients, even when they have a concentration of serum methylmalonic acid below the upper reference limit. It is possible that the two enzymes that require cobalamin, methionine synthase (which requires methyl-cobalamin) and methylmalonyl-CoA mutase (which requires adenosyl-cobalamin), behave differently in their response to the declining availability of cobalamin. Possibly the two enzymes have different affinities for their respective forms of cobalamin, or there is a difference in the availability of the two forms of cobalamin. The concentration of serum methylmalonic acid might, therefore, not reflect the tissue cobalamin availability for methionine synthase.

There are several possible explanations for the association between increased plasma tHcy and dementia. Several studies (33) have shown that homocysteine is associated with an increased risk of vascular diseases. Both cardiovascular disease and carotid atherosclerosis have been related to cognitive impairment and dementia (34–36). Additionally, homocysteine could be associated with cognitive function through other mechanisms, such as a direct neurotoxic effect (10, 16). Furthermore, increased plasma tHcy is a marker of cobalamin/folate deficiency. The metabolism of folate and cobalamin is connected in one reaction, the remethylation of homocysteine to methionine catalyzed by the cobalamin- and folate-dependent enzyme methionine synthase (10). S-Adenosylmethionine is subsequently formed. This metabolite is involved in numerous methylation reactions that involve proteins, phospholipids, DNA, and neurotransmitter metabolism (1). It has been suggested that a defect in methylation processes is central to the neuropsychiatric manifestations of these vitamin deficiencies (1). Both folate and cobalamin deficiencies may cause similar neurological and psychiatric disturbances, including depression, aggravation of dementia, and a demyelinating myelopathy. The most common finding associated with cobalamin deficiency was peripheral neuropathy, whereas folate deficiency was associated mainly with depression (3).

In conclusion, the findings presented in this study show that the concentrations of serum cobalamin and methylmalonic acid do not reflect the functional indicators of the central nervous system, the impaired function of the remethylation pathway may be associated with a marginal folate deficiency. The findings suggest that of the markers measured in the present study, plasma tHcy is the best marker to measure in suspected cobalamin/folate deficiency.

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References


