Increased Plasma Methylmalonic Acid Level Does Not Predict Clinical Manifestations of Vitamin B₁₂ Deficiency

Anne-Mette Hvas, MD; Jørgen Ellegaard, MD; Ebba Nexø, MD

Background: The prevalence of vitamin B₁₂ deficiency, defined as an elevated concentration of plasma methylmalonic acid (P-MMA), has been estimated to be 15% to 44% in the elderly. However, we do not know whether an increased P-MMA level actually indicates or predicts a clinical condition in need of treatment.

Participants and Methods: In a follow-up study, 432 individuals not treated with vitamin B₁₂ were examined 1.0 to 3.9 years after initial observation of an increased P-MMA concentration (>0.28 µmol/L). The examination included laboratory tests, a structured interview to disclose symptoms, a food frequency questionnaire, and a clinical examination including a Neurological Disability Score.

Results: Variation in P-MMA levels over time was high (coefficient of variation, 34%). In only 16% of participants, P-MMA levels increased substantially, whereas 44% showed a decrease. Level of P-MMA was significantly but not strongly associated with levels of plasma cobalamins (r = -0.22, P < .001) and plasma total homocysteine (r = 0.37, P < .001). After adjustment for age and sex, we found no associations between P-MMA concentration and the total symptom score (P = .61), the total Neurological Disability Score (P = .64), or other clinical manifestations related to vitamin B₁₂ deficiency.

Conclusions: An increased level of P-MMA did not predict a further increase with time and clinical manifestations related to vitamin B₁₂ deficiency. We therefore challenge the use of an increased P-MMA concentration as the only marker for diagnosis of vitamin B₁₂ deficiency.

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The original concept of pernicious anemia, defined as lack of intrinsic factor, represents only one possible and rather rare presentation of vitamin B₁₂ deficiency. Strong incentives exist to establish accurate diagnostic tests because of the often diffuse and nonspecific symptoms of mild vitamin B₁₂ deficiency. Anemia might be absent and damage to the nervous system might be reversible when treated in time but irreversible after delayed diagnosis.

Use of the deoxyuridine suppression test has permitted recognition of early and mild vitamin B₁₂ deficiency characterized by biochemical dysfunction but lack of clear clinical features of deficiency. However, the test has limited clinical applicability because it is cumbersome to perform. During the past 10 years, determination of plasma methylmalonic acid (P-MMA) and plasma total homocysteine (P-tHcy) levels has been increasingly used. Level of P-MMA has been suggested as a more specific and sensitive marker than levels of plasma cobalamins. Prevalence estimates of vitamin B₁₂ deficiency, defined as an elevated P-MMA concentration, vary widely. Studies from the United States suggest a prevalence of 15% to 20% among elderly outpatients (P-MMA level >0.37 µmol/L), whereas European studies suggest a prevalence of 39% to 44% among healthy elderly individuals (P-MMA level >0.24 µmol/L), and a prevalence of 24% among free-living elderly Dutch persons (P-MMA level >0.32 µmol/L). However, it is now uncertain to which extent an increase in the P-MMA level actually indicates or predicts a clinical condition in need of treatment, and we still lack consensus about a gold standard for the diagnosis of vitamin B₁₂ deficiency.

In the present study we questioned the clinical significance of an increased P-MMA level. The study aims were to estimate the long-term trend of an initially elevated P-MMA level in individuals who did not receive cyanocobalamin therapy and to examine the associations between clinical manifestations related to vitamin B₁₂ deficiency and elevated
PARTICIPANTS AND METHODS

STUDY POPULATION

From the laboratory information system (Department of Clinical Biochemistry, Skejby Sygehus, Aarhus University Hospital, Aarhus, Denmark) we obtained information on 1754 individuals aged 18 years and older living in the Aarhus municipality (283000 inhabitants) who had a P-MMA level greater than the reference interval (>0.28 µmol/L) between January 1, 1995, and December 31, 1997 (prestudy P-MMA) (Figure 1). Measurement of P-MMA concentration was requested by the physician in charge of the patient because of suspected vitamin B12 deficiency.

To exclude individuals who had received cyanocobalamin treatment we used a 3-step procedure. From National Health Insurance, Aarhus County, we received information on all prescribed cyanocobalamin preparations. For all individuals not excluded by this procedure we asked their general practitioner about cyanocobalamin prescriptions. Finally, the initial interview included questions about previous and current treatment with cyanocobalamin.

A total of 371 individuals (33%) had received cyanocobalamin treatment and were excluded, and another 28 individuals were excluded because they had participated in a preceding pilot study.

Of 1155 individuals with no report of cyanocobalamin treatment, we included all 336 with prestudy P-MMA levels of 0.40 µmol/L or greater and took a geographical sample of 647 individuals from 819 with prestudy P-MMA levels of 0.29 to 0.39 µmol/L.

Of the 983 individuals addressed, 49 reported that they had received cyanocobalamin treatment, 21 had died, and 1 had emigrated, leaving 912 individuals eligible for follow-up examination and 19 reported during the interview that they had received cyanocobalamin treatment. The follow-up examinations of the 432 participants were performed between October 7, 1998, and May 31, 1999, 1.0 to 3.9 years after the prestudy P-MMA measurement.

The study was approved by the Research Ethics Committee of Aarhus County. Written informed consent was obtained from all participants.

LABORATORY TESTS

Levels of P-MMA were measured using stable isotope-dilution capillary gas chromatography–mass spectrometry (analytical imprecision <8%); the reference interval was 0.08 to 0.28 µmol/L.21

Levels of P-tHcy were measured using an immunological method and Imx (Abbott Laboratories, Abbott Park, Ill) equipment (analytical imprecision <5%). Plasma was separated from the blood cells within 2 hours. The reference interval was 5.8 to 11.9 µmol/L. Levels of plasma cobalamin were determined using an automated chemiluminescence system (ACS: Centaur Automated Chemiluminescence System; Chiron Diagnostics Corporation, East Walpole, Mass) and a competitive protein binding assay (analytical imprecision <10%); the reference interval was 200 to 6000 pmol/L.

Standard methods were used for determination of hematologic parameters. Reference intervals for blood hemoglobin levels were 7.40 to 9.60 mmol/L for women and 8.40 to 10.80 mmol/L for men and for erythrocyte mean cell volume was 85 to 100 fL. Plasma creatinine level was measured using the Jaffe method and a Roche Cobas Integra 700 autoanalyzer (HiCo Creatinine Jaffe method; Boehringer Mannheim GmbH, Mannheim, Germany) (analytical imprecision <3%); the reference intervals were 44 to 115 µmol/L (0.5-1.3 mg/dL) for women and 62 to 133 µmol/L (0.7-1.5 mg/dL) for men.

INTERVIEW AND CLINICAL EXAMINATIONS

A history of present and previous diseases was obtained. Information on symptoms was obtained by structured interview. We recorded anemia symptoms (daily fatigue, palpitations, shortness of breath, and angina on effort), gastrointestinal symptoms (reduced sense of taste, sore mouth or tongue, daily reduced appetite, daily nausea, and daily diarrhea), and neurological symptoms using a slightly modified version of the Neurological Symptom Score.21 Anemia, gastrointestinal, and Neurological Symptom Scores were summed to a total symptom score. In addition, we recorded current drug use and consumption of alcohol. Dietary vitamin B12 intake was estimated using part of a validated food frequency questionnaire.23-25

The neurological examination comprised testing for vibration sense, joint position sense, cutaneous sensation, hyporeflexia, and muscular strength. Vibration sense was tested at the medial malleolus, compared with a stimulus at the processus styloideus ulnae. Joint position sense was tested at the hallux and the index finger. Cutaneous sensation was tested by pinprick on the pulp of the hallux and the index finger and by light touching of the dorsum of the foot, the shin, and the forearm. A test for the Romberg sign was performed and gait was assessed. “Finger-nose” and “heel-knee-shin” tests were performed, as was testing for dyssyadiadochokinesis.

We used a slightly modified version of the Neurological Disability Score (a summed score of muscle strength, reflexes, and sensory loss) to quantify the degree of peripheral neuropathy.21 The Neurological Disability Score was the sum of 28 item scores, each ranging from 0 (normal) to 4 (high degree of impairment).

In addition, the examination included assessment of the nutritional state, inspection of the oral cavity, heart and lung auscultation, blood pressure measurement, and abdominal palpation.

All participants were examined by the same investigator (A.-M.H.), who did not know the laboratory test results when the examinations were performed.

STATISTICAL ANALYSIS

For analyses of associations among laboratory test results we used the t test (independent samples), the χ2 test for trend, linear regression, the Pearson correlation, and the Levene test. To analyze the associations between the biochemical markers and the clinical manifestations we used linear and logistic regression. Log transformations were used when appropriate. Differences were regarded as statistically significant at P<.05. Data were entered and analyzed using statistical analysis software (SPSS for Windows; SPSS Inc, Chicago, Ill).
Association between prestudy and follow-up plasma methylmalonic acid (P-MMA) levels in 432 individuals with a prestudy P-MMA concentration (median, 0.28 µmol/L) who were not treated with cyanocobalamin before measurement of P-MMA levels 1.0 to 3.9 years later.

Table 1. Association Between Prestudy P-MMA Levels and the Change in P-MMA Levels During Follow-up*

<table>
<thead>
<tr>
<th>Prestudy P-MMA, µmol/L</th>
<th>&gt;20% Decrease</th>
<th>&gt;20% Change</th>
<th>&gt;20% Increase</th>
<th>Total†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.29-0.39</td>
<td>138 (45)</td>
<td>128 (41)</td>
<td>44 (14)</td>
<td>310 (100)</td>
</tr>
<tr>
<td>≥0.40</td>
<td>54 (44)</td>
<td>41 (34)</td>
<td>27 (22)</td>
<td>122 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>192 (44)</td>
<td>169 (39)</td>
<td>71 (16)</td>
<td>432 (100)</td>
</tr>
</tbody>
</table>

* P-MMA indicates plasma methylmalonic acid.
† Not all percentages equaled exactly 100.

The interval from the prestudy measurement of P-MMA to follow-up was 1.0 to 1.9 years for 59% of participants, 2.0 to 2.9 years for 24%, and 3.0 to 3.9 years for 17%. Figure 2 shows the association between prestudy and follow-up P-MMA levels. The correlation between the log-transformed measurements was significant (P < .001), but the variation was substantial (coefficient of variation, 34%, estimated from the SD of the log-transformed ratio, follow-up vs prestudy P-MMA levels). The coefficient of determination (R²) was 0.24, indicating that only 24% of the variation in follow-up P-MMA levels could be explained by the variation in prestudy P-MMA levels.

Table 1 shows the association between prestudy P-MMA levels and the change in P-MMA levels during follow-up. In general, P-MMA levels did not increase; only 16% of participants had an increase of more than 20%, whereas 45% had a decrease of more than 20%. Only 13 participants (3%) had a P-MMA concentration of 1.00 µmol/L or greater at follow-up. The duration of follow-up did not affect the magnitude of change (P = .72, χ² for trend).

The variation in the ratio of follow-up vs prestudy P-MMA levels was significantly higher in participants with...
presurvey P-MMA levels of 0.40 µmol/L or greater than in participants with lower presurvey P-MMA levels (P<.001, log-transformed data, Levene test).

Plasma creatinine concentration was known at the time of the presurvey P-MMA measurement for 110 participants. No correlation was found between change in P-MMA level and change in plasma creatinine level (r = 0.12; P = .21, log-transformed data).


Table 2 shows the distribution of age and the test values in the study group. Plasma creatinine level is included because it is important for the interpretation of P-MMA levels.\(^\text{26,27}\)

Using linear regression analysis we found strong positive associations between P-MMA level and age, P-tHcy level and age, and plasma creatinine level and age (P<.001 for all), but no association was found between the level of plasma cobalamins and age (P = .16).

Significant but not strong associations in the expected directions were found between the markers of vitamin B\(_12\) deficiency: levels of P-MMA and plasma cobalamins, r = −0.22; P < .001, log-transformed data; and levels of P-MMA and P-tHcy, r = 0.37; P < .001, log-transformed data. The associations were strong between levels of P-MMA as well as P-tHcy and plasma creatinine levels (Figure 3) and remained when controlled for confounding by age.

Using linear regression analysis, P-MMA level was weakly, but significantly, associated with blood hemoglobin level (P < .001) and erythrocyte mean cell volume (P = .01) (Figure 4). However, we found no difference in blood hemoglobin level (P = .94) or erythrocyte mean cell volume (P = .96) comparing participants with a 20% increase in P-MMA level with those with a 20% decrease in P-MMA level. Sixty participants (14%) had anemia, and a subgroup of 14 participants had blood hemoglobin levels below the reference interval and erythrocyte mean cell volumes greater than 95 fl. In this subgroup, no association was found among low blood hemoglobin levels, increased erythrocyte mean cell volumes, and levels of P-MMA (P = .93, linear regression adjusted for age, sex, and plasma creatinine level).

**CLINICAL MANIFESTATIONS**

Of 403 participants who underwent clinical examination, 397 (99%) were of Danish origin, 249 (62%) were women, and 13 (3%) were living in institutions. Twenty-five participants (6%) had diabetes, 15 (4%) had hypothyroidism, and 134 (33%) recorded cardiovascular disease.

The clinical manifestations were evaluated as symptoms (complaints reported by the participants) and signs (manifestations recorded by the examining physician).

**Symptoms**

Symptoms possibly related to vitamin B\(_12\) deficiency were prevalent: 113 participants (28%) had more than 1 neurological symptom, 243 (60%) had at least 1 symptom compatible with anemia, and 127 (32%) had at least 1 gastrointestinal symptom. Figure 5 shows a weak association between P-MMA concentration and the prevalence of neurological symptoms, and a stronger association with age. When adjusting for age and sex, no association was found between prestudy P-MMA levels and symptom scores of anemia (P = .68), neurological (P = .56), or gastrointestinal (P = .76) symptoms or the total symptom score (P = .61). Neither did we find any associations between follow-up levels of P-MMA, P-tHcy, or plasma cobalamins and symptom scores (Table 3). Adjustment for plasma creatinine level did not alter the results (data not shown).

Presuming that vitamin B\(_12\) deficiency is a likely diagnosis when levels of P-MMA and plasma cobalamins are abnormal or levels of P-MMA and P-tHcy are abnormal, we compared symptom scores in participants having 2 abnormal test results with those having 2 normal test results. Still, in these analyses, we found no association between the biochemical markers and symptom scores.

We examined whether participants with an increase in P-MMA concentration of more than 20% differed from participants with a decrease of more than 20% between prestudy and follow-up. No association was found between change in P-MMA level and the prevalence of symptoms (linear regression adjusted for age and

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*P-MMA indicates plasma methylmalonic acid; P-tHcy, plasma total homocysteine.
†To convert plasma creatinine from micromoles per liter to milligrams per deciliter, divide micromoles per liter by 88.4.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>5</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>95</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prestudy P-MMA, µmol/L</td>
<td>0.29</td>
<td>0.29</td>
<td>0.31</td>
<td>0.33</td>
<td>0.41</td>
<td>0.76</td>
<td>3.60</td>
</tr>
<tr>
<td>Follow-up P-MMA, µmol/L</td>
<td>0.06</td>
<td>0.14</td>
<td>0.23</td>
<td>0.30</td>
<td>0.41</td>
<td>0.75</td>
<td>5.3</td>
</tr>
<tr>
<td>Plasma cobalamins, pmol/L</td>
<td>78</td>
<td>147</td>
<td>211</td>
<td>272</td>
<td>354</td>
<td>538</td>
<td>1549</td>
</tr>
<tr>
<td>P-tHcy, µmol/L</td>
<td>3.43</td>
<td>6.27</td>
<td>8.95</td>
<td>11.36</td>
<td>15.31</td>
<td>24.21</td>
<td>68.76</td>
</tr>
<tr>
<td>Plasma creatinine, µmol/L</td>
<td>50</td>
<td>66</td>
<td>80</td>
<td>90</td>
<td>105</td>
<td>153</td>
<td>584</td>
</tr>
<tr>
<td>Blood hemoglobin, mmol/L</td>
<td>5.70</td>
<td>7.30</td>
<td>8.10</td>
<td>8.70</td>
<td>9.20</td>
<td>10.20</td>
<td>11.60</td>
</tr>
<tr>
<td>Erythrocyte mean cell volume, fL</td>
<td>72</td>
<td>84</td>
<td>89</td>
<td>92</td>
<td>95</td>
<td>102</td>
<td>118</td>
</tr>
<tr>
<td>Age, y</td>
<td>23.9</td>
<td>34.1</td>
<td>56.5</td>
<td>72.2</td>
<td>80.2</td>
<td>87.6</td>
<td>102.6</td>
</tr>
</tbody>
</table>

CLINICAL MANIFESTATIONS

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†To convert plasma creatinine from micromoles per liter to milligrams per deciliter, divide micromoles per liter by 88.4.
Finally, no significant difference in prevalence of symptoms was found between participants with P-MMA levels permanently greater than or equal to 0.40 µmol/L (n=60) and those whose levels were permanently less than 0.40 µmol/L (n=256).

Signs

The maximum Neurological Disability Score was 112 points. Eighty-three participants (21%) had a normal score of zero and 148 (37%) had a score of more than 10 points. Figure 6 shows the distribution of Neurological Disability Scores at different levels of P-MMA and in different age groups. The prevalence of a high Neurological Disability Score did not increase much with a higher level of P-MMA, whereas age and Neurological Disability Score were associated.

Using linear regression adjusted for age and sex we found a significant but weak association between the prestudy P-MMA level and the total Neurological Disability Score ($r=0.10; P=0.05$, log-transformed data). No association was found between the follow-up P-MMA level and the total Neurological Disability Score ($P=0.64$, log-transformed data). Furthermore, the Neurological Disability Scores of participants having 2 abnormal test results did not differ from those having 2 normal test results. Participants with a P-MMA increase of more than 20% or 50% did not have an increased Neurological Disability Score compared with other participants.

No associations were found between P-MMA level and nutritional state or neurological signs (Table 3). Neither did we find any significant associations between levels of P-tHcy or plasma cobalamins and the recorded signs. The results were essentially unchanged after adjustment for plasma creatinine level (data not shown).
VITAMIN SUPPLEMENTATION

One hundred eighteen participants took vitamin supplements daily typically containing 1 to 2 µg of cyanocobalamin and 200 µg of folic acid. We found a significant inverse association between intake of vitamin supplements and P-tHcy level (P=.002, linear regression adjusted for age and sex). This association was not found for levels of P-MMA or plasma cobalamins. We did not find any association between estimates of vitamin B12 intake from food and levels of P-MMA, P-tHcy, and plasma cobalamins (data not shown).

We studied 432 individuals not treated for vitamin B12 deficiency despite an increased concentration of P-MMA. We report a large variation in P-MMA levels over time. Furthermore, we found no association between the concentration of P-MMA and clinical manifestations related to vitamin B12 deficiency.

It is relatively easy to diagnose overt vitamin B12 deficiency, but to diagnose mild vitamin B12 deficiency is difficult. If an elevated P-MMA level reflects a chronic or progressive condition, we would expect an increased P-MMA level to be stable or to increase further over time in individuals not treated with cyanocobalamin. The variation between the prestudy and follow-up P-MMA levels was considerable (coefficient of variation, 34%), indicating that the prestudy P-MMA level only contributes little to the prediction of the P-MMA level at follow-up. In general, we did not find an increase in the P-MMA level measured 1.0 to 3.9 years after the initially increased level. An increased P-MMA level that normalizes on treatment with cyanocobalamin has been suggested as a diagnostic test.2,10,11,28-30 Our results question this diagnostic criterion because almost half the patients showed a decrease of more than 20% in P-MMA concentration over time without cyanocobalamin treatment. The average decrease in P-MMA concentration can partly be explained by regression toward the mean, but still the trend is remarkable.

In the present study we examined the clinical correlates of abnormal levels of P-MMA, P-tHcy, and plasma cobalamins. We used a structured interview to assess the symptoms, which allowed us to quantify neurological and gastrointestinal symptoms as well as symptoms of anemia. To assess neuromuscular dysfunction we chose the Neurological Disability Score, in which selected items from the conventional neurological examination are scored.22 This method is considered useful,31 but it has low sensitivity and might not be as objective or reproducible as desirable.

Although the symptoms and signs related to vitamin B12 deficiency are not specific, we expected an association between the biochemical markers and the clinical manifestations. The associations found were insignificant, weak, and in shifting directions. We found that age was a strong predictor for symptoms and signs, whereas levels of P-MMA, P-tHcy, and plasma cobalamins did not add further to the prediction of clinical manifestations.

The concentration of P-MMA might be affected by conditions other than vitamin B12 deficiency. Renal failure is considered the most important condition,10,27,32 but intravascular volume depletion,33 changes in propionic acid–producing bacteria in the gut flora,8 pregnancy,34 and thyroid disease35 might also affect the P-MMA level. In our study, renal failure was the most likely confounder. However, our results remained essentially unchanged after controlling for plasma creatinine level.

Participants were identified by the laboratory information system, implying some selection as they were seen by the general practitioner or hospitalized when the pre-study P-MMA level was measured. They thus represent individuals suspected of having vitamin B12 deficiency. We are confident that the 3-step procedure to identify individuals who had received cyanocobalamin treatment was efficient and that the findings were not confounded by the effect of treatment. However, the associations between biochemical findings and clinical manifestations might be affected by selection to treatment of individuals with typical symptoms, leaving individuals with less pronounced symptoms for this study of the untreated. We cannot dismiss this selection bias; however, in a previous study30 of physicians’ reactions to an increased concentration of P-MMA we found that only 22% of patients with an increased P-MMA level were selected for treatment and the remaining were not treated. Treated patients did not differ from the untreated in clinical manifestations.

Based on our present results we disagree with authors who suggest that P-MMA is a useful variable for screening the elderly for vitamin B12 deficiency.37-39 Furthermore, we do not recommend use of an increased P-MMA concentration as the sole indicator for starting lifelong cyanocobalamin treatment. If no other symptoms or signs indicate vitamin B12 deficiency, we suggest patients be followed up later rather than initiating treatment for vitamin B12 deficiency immediately. However, the relatively weak correlation between P-MMA and P-tHcy levels might well be explained by the fact that the P-tHcy level increases also in patients with folate or vitamin B6 deficiency. Hyperhomocysteinemia has recently been
Table 3. Associations Between the Biochemical Markers and Symptoms and Signs of Vitamin B<sub>12</sub> Deficiency, Adjusted for Age and Sex, in 403 Participants Who Underwent Clinical Examination<sup>a</sup>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>P-MMA</th>
<th>P-tHcy</th>
<th>Plasma Cobalmins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms of anemia†</td>
<td>.31‡</td>
<td>.15</td>
<td>&gt; .99</td>
</tr>
<tr>
<td>Neurological symptoms‡</td>
<td>.73</td>
<td>.50</td>
<td>.57</td>
</tr>
<tr>
<td>Gastrointestinal symptoms†</td>
<td>.15‡</td>
<td>.01</td>
<td>.67‡</td>
</tr>
<tr>
<td>Total symptom score†</td>
<td>.81‡</td>
<td>.11</td>
<td>.78</td>
</tr>
<tr>
<td>Signs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurological Disability Score†</td>
<td>.64‡</td>
<td>.62</td>
<td>.39</td>
</tr>
<tr>
<td>Nutritional state§</td>
<td>0.87 (0.43-1.76)</td>
<td>1.84 (0.73-4.68)</td>
<td>0.83 (0.35-1.94)</td>
</tr>
<tr>
<td>Insecure “finger-nose” test§</td>
<td>0.78 (0.47-1.29)</td>
<td>1.08 (0.55-2.12)</td>
<td>1.67 (0.93-3.01)</td>
</tr>
<tr>
<td>Insecure “heel-knee-shin” test§</td>
<td>1.03 (0.62-1.71)</td>
<td>1.23 (0.61-2.47)</td>
<td>1.09 (0.60-1.98)</td>
</tr>
<tr>
<td>Dysdiadochokinesis§</td>
<td>0.43 (0.14-1.29)</td>
<td>0.99 (0.27-3.60)</td>
<td>1.23 (0.42-3.60)</td>
</tr>
<tr>
<td>Present Romberg sign§</td>
<td>0.67 (0.40-1.13)</td>
<td>1.04 (0.53-2.07)</td>
<td>1.03 (0.57-1.87)</td>
</tr>
<tr>
<td>Abnormal gait§</td>
<td>1.09 (0.65-1.83)</td>
<td>1.96 (0.97-3.99)</td>
<td>0.81 (0.45-1.47)</td>
</tr>
</tbody>
</table>

<sup>a</sup>P-MMA indicates plasma methylmalonic acid; P-tHcy, plasma total homocysteine.
† Linear regression analysis. Data are given as P values.
‡ The direction of the association was opposite that expected.
§ Logistic regression analysis. Data are given as odds ratios per unit change in the log-transformed biochemical markers (95% confidence intervals).

Figure 6. Neurological Disability Scores (NDS) by level of plasma methylmalonic acid (P-MMA) and age group in 403 individuals not treated with cyanocobalamin.

linked to cardiovascular disease,<sup>40</sup> but it is still discussed whether treatment or prevention with B vitamins will reduce the cardiovascular risk.<sup>41</sup>

The lack of association between clinical manifestations and biochemical indices of vitamin B<sub>12</sub> deficiency might be attributable to the high prevalence of symptoms of anemia and neurological abnormalities in the elderly. We expect that individuals with clinical manifestations and abnormal values for one or more of the biochemical markers would benefit from treatment with cyanocobalamin, but at present we do not know whether this is the case. A randomized clinical study is needed to answer this important question.

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Corresponding author and reprints: Anne-Mette Hvas, MD, Department of Hematology, Aarhus University Hospital, Aarhus Amtssygehus, Tage Hansens Gade 2, 8000 Aarhus C, Denmark (e-mail: Anne_Mette.Hvas@aaauh.dk).

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