Table 1. Intraassay variation and recovery of 5,6-dihydrouracil in urine, filter paper extracts, and plasma.\textsuperscript{a}

\begin{tabular}{|c|c|c|c|c|c|}
\hline
 & \textbf{Intraassay variation} & \textbf{Recoveries}\textsuperscript{b} & \\
 & \textbf{(n = 10)} & \textbf{\(\mu\text{mol/L}\)} & \textbf{CV, \%} & \textbf{\(\mu\text{mol/L}\)} & \textbf{Recovery, \%} & \textbf{CV, \%} \\
\hline
\textbf{Urine} & & & & & & \\
Blank & 1.0 (0.9) & 89 & 8.8 (5.3) & 60 & \\
Low & 11.7 (1.0) & 8 & 103 (31) & 31 & \\
Medium & 100 (4) & 4 & 97 (6) & 6 & \\
High & 1042 (50) & 5 & 103 (9) & 9 & \\
\hline
\textbf{Urine-filter paper} & & & & & & \\
Blank & ND & 12 (8.7) & 73 & \\
Low & 11.8 (2.9) & 25 & 81 (42) & 51 & \\
Medium & 102 (7) & 7 & 89 (9) & 10 & \\
High & 1016 (58) & 6 & 96 (12) & 12 & \\
\hline
\textbf{Plasma} & & & & & & \\
Blank & 1.2 (0.3) & 22 & 1.3 (0.6) & 46 & \\
Low & 2.1 (0.4) & 21 & 101 (83) & 82 & \\
Medium & 11 (1) & 9 & 103 (7) & 7 & \\
High & 102 (3) & 3 & 101 (6) & 6 & \\
\hline
\end{tabular}

\textsuperscript{a}Values in parentheses are the SD. \\
\textsuperscript{b}Recoveries in 10 different urine and plasma samples. \\
\textsuperscript{c}Supplemented with 1 \(\mu\text{mol/L}\) (plasma) or 10 \(\mu\text{mol/L}\) (urine). \\
\textsuperscript{d}Supplemented with 10 \(\mu\text{mol/L}\) (plasma) or 100 \(\mu\text{mol/L}\) (urine). \\
\textsuperscript{e}Supplemented with 100 \(\mu\text{mol/L}\) (plasma) or 1000 \(\mu\text{mol/L}\) (urine). \\
\textsuperscript{f}ND, not detectable (<2 \(\mu\text{mol/L}\)).

References


DOI: 10.1373/clinchem.2003.026229

Vitamin B12 Status in the Elderly as Judged by Available Biochemical Markers, Rima Obeid,\textsuperscript{1} Heike Schorr,\textsuperscript{2} Rudolf Eckert,\textsuperscript{2} and Wolfgang Herrmann\textsuperscript{1*} (1 Department of Clinical Chemistry/Central Laboratory, Saarland University Hospital, Homburg, Germany; \textsuperscript{2}Geriatric Rehabilitation Clinic, St. Ingbert, Germany; \textsuperscript{*}address correspondence to this author at: Zentrallabor der Universitätskliniken des Saarlandes, Kirrberger Strasse, Gebäude 40, 66421 Homburg, Germany; fax 49-6841-1623109, e-mail kchwhe@uniklinik-saarland.de)

Deficiencies of vitamin B12, B6, and folate are frequent in elderly individuals (1–4), with estimated prevalences in elderly individuals of 5–40% depending on the marker for deficiency and the cutoff used (1–4). Detection of a subnormal status of these B vitamins has considerable importance. Vitamin B12 deficiency in the elderly may cause cognitive dysfunction (5), neuropsychiatric disorders, dementia (6), and hyperhomocysteinemia (5), an independent risk factor for coronary vascular disease and dementia (7, 8). A 5 \(\mu\text{mol/L}\) increment in total plasma homocysteine (tHcy) has been associated with a 49% increase in all-cause of mortality among individuals 65–72 years of age (9).

The measurement of total vitamins in serum has a limited sensitivity and specificity, especially in individuals with serum vitamin B12 <300 \(\mu\text{mol/L}\) (1, 3). More specific and sensitive biochemical approaches have been developed recently (10, 11). Among the biochemical markers whose concentrations are increased in vitamin B12-deficient individuals are methylmalonic acid (MMA) and tHcy, which is also increased in deficiencies of folate and vitamin B6 (1, 10).

Increased MMA and tHcy have been observed in elderly individuals in spite of serum concentrations of the B vitamins within the appropriate reference intervals (1, 2). High concentrations of these metabolites were decreased by supplementation with B vitamins, which suggests pretreatment deficiency (12–14). The current study was undertaken to investigate vitamin B12 status in a group of elderly Germans, as determined by some available biochemical markers.

The study population comprised 228 individuals > 65 years of age. We recruited 109 free-living elderly individuals [mean (SD) age, 79 (10) years] through use of a general practitioner’s register and 119 elderly individuals [82 (6) years] who were institutionalized for 6 weeks for recovery from various illnesses. All were in good general condition with no acute diseases. Exclusion criteria included vitamin consumption, life-threatening illness, serious malignancies, renal insufficiency, recent myocardial infarction, or cerebrovascular event. Diabetes mellitus, hypertension, chronic heart failure, and history of coronary vascular disease were found in 18%, 38%, 43%, and 26%, respectively. A group of apparently healthy younger adults who did not take vitamins [n = 74; mean (SD) age, 43 (17) years] was also studied.

Fasting blood was drawn into tubes without anticoagulant. Blood samples were immediately placed on ice and centrifuged within 45 min at 2000 \(\text{g}\) and 4 °C. Serum was separated and stored at −70 °C for further analyses. Serum concentrations of tHcy, MMA, and cystathionine were measured by gas chromatography–mass spectrometry as described elsewhere (1). The CV for tHcy, MMA, and cystathionine assays were 3.2%, 5.3%, and 5.8%, respectively. Serum concentrations of folate and vitamin B12 were measured by a chemiluminescence immunoassay (ADVIA Centaur; Bayer). The concentration of holoTC was assayed by RIA (Axis-Shift) (15). The CV for holoTC assay were 8.0% and 5.0% at 38 and 98 \(\mu\text{mol/L}\), respectively. Serum concentrations of vitamin B6 (pyridoxal 5-phosphate) were measured by
The data analyses were performed with SPSS (Ver. 11.0 for Windows). Categorical variables were compared by the χ² test. One-way ANOVA and the post hoc Tamhane-T2 tests were applied for multiple comparisons. The odds ratios for pathologically increased or decreased markers among two ranges of serum creatinine were calculated by logistic regression analysis. Logarithmic transformation was applied where appropriate. Correlations between different markers were assessed by the Spearman test. P values <0.05 were regarded as significant. Increased concentrations of the metabolites or a decreased concentration of the vitamin was considered as that ≥95% or ≤5%, respectively, of the data in the younger individuals. Increased serum creatinine was considered as >79.6 and 106.1 μmol/L for females and males, respectively.

The concentrations of the metabolites and the B vitamins are shown in Table 1 according to age. The concentrations of tHcy, MMA, and cystathionine were highest in individuals >80 years. Individuals >80 years had the lowest median vitamin B₁₂, B₆, and folate concentrations. Median holoTC was only slightly lower in individuals >80 years compared with those ≤65 years. The incidence of increased concentrations of the metabolites increased with age, whereas the prevalence of a decreased holoTC was only slightly increased with increasing age (Table 1). Median concentrations of tHcy, holoTC, and vitamin B₁₂ are shown according to age and intergroup quartiles of MMA (Fig. 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue1/).

Compared with institutionalized elderly, free-living elderly individuals were older (median age, 81 vs 74 years; P = 0.014) and had lower vitamin B₁₂ (median, 251 vs 270 pmol/L; P = 0.006) and lower serum folate (13.0 vs 14.3 nmol/L; P = 0.003).

Among individuals >65 years, the odds ratio for a tHcy ≥14.1 μmol/L was 2.8 (95% confidence interval, 1.6–4.9) for individuals with high serum creatinine compared with individuals with serum creatinine within the reference interval. The likelihoods of finding MMA ≥0.28 μmol/L or holoTC ≤29 pmol/L were 2.4 (95% confidence interval, 1.4–4.3) and 0.6 (95% confidence interval, 0.3–1.2), respectively, in individuals with high serum creatinine. Elderly individuals with creatinine within the reference interval had similar incidences of increased MMA and decreased holoTC (25% vs 18%). Increased MMA and decreased holoTC were detected in 45% and 11%, respectively, of elderly individuals with increased creatinine.

We calculated the quartiles of holoTC and folate in two age groups (≤65 and >65 years; Fig. 1). MMA was also within reference values in individuals >65 years who had creatinine within the reference interval and holoTC ≥37 pmol/L and in elderly with increased creatinine who had holoTC ≥54 pmol/L (Fig. 1A). Younger adults had a mean MMA concentration <0.28 μmol/L in all quartiles of holoTC (Fig. 1B). Elderly individuals with creatinine within the reference interval had a mean tHcy <14.1 μmol/L when serum folate was >13.5 nmol/L (Fig. 1C). Mean tHcy was increased in individuals with increased creatinine in all quartiles of serum folate.

Table 1. Characteristics of the study participants according to age.

<table>
<thead>
<tr>
<th>Characteristic or marker</th>
<th>21–65 years</th>
<th>66–80 years</th>
<th>&gt;80 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>74</td>
<td>110</td>
<td>118</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>42 (57%)</td>
<td>66 (59%)</td>
<td>90 (76%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>51 (21–65)</td>
<td>74 (66–80)</td>
<td>87 (81–99)</td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>9.3 (4.6–20.5)</td>
<td>13.0 (6.7–36.6)</td>
<td>15.8 (7.7–43.5)</td>
</tr>
<tr>
<td>tHcy ≥14.1 μmol/L, %</td>
<td>5</td>
<td>43%</td>
<td>67%</td>
</tr>
<tr>
<td>MMA, μmol/L</td>
<td>0.16 (0.07–0.45)</td>
<td>0.20 (0.07–1.35)</td>
<td>0.24 (0.09–4.58)</td>
</tr>
<tr>
<td>MMA ≥0.28 μmol/L, %</td>
<td>5</td>
<td>28%</td>
<td>39</td>
</tr>
<tr>
<td>Cystathionine, nmol/L</td>
<td>218 (101–676)</td>
<td>284 (114–2774)</td>
<td>473 (169–8040)</td>
</tr>
<tr>
<td>Cystathionine ≥437 nmol/L</td>
<td>5</td>
<td>30%</td>
<td>56%</td>
</tr>
<tr>
<td>Vitamin B₁₂, pmol/L</td>
<td>283 (151–728)</td>
<td>274 (101–1983)</td>
<td>254 (70–2445)</td>
</tr>
<tr>
<td>Vitamin B₁₂ ≥196 pmol/L, %</td>
<td>5</td>
<td>22%</td>
<td>30%</td>
</tr>
<tr>
<td>HoloTC, pmol/L</td>
<td>53 (11–196)</td>
<td>62 (4–320)</td>
<td>47 (7–320)</td>
</tr>
<tr>
<td>HoloTC ≥29 pmol/L, %</td>
<td>5</td>
<td>11%</td>
<td>20%</td>
</tr>
<tr>
<td>Folate, nmol/L</td>
<td>20.6 (9.3–79.7)</td>
<td>13.9 (5.7–161.0)</td>
<td>13.4 (4.1–61.2)</td>
</tr>
<tr>
<td>Folate ≥11.1 nmol/L, %</td>
<td>5</td>
<td>25%</td>
<td>28%</td>
</tr>
<tr>
<td>Vitamin B₆, nmol/L</td>
<td>52 (9–229)</td>
<td>28 (2–278)</td>
<td>21 (2–120)</td>
</tr>
<tr>
<td>Vitamin B₆ &lt;19.3 nmol/L, %</td>
<td>5</td>
<td>29%</td>
<td>44%</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>70.7 (42.4–114.9)</td>
<td>88.4 (53.0–194.5)</td>
<td>88.4 (44.2–185.6)</td>
</tr>
</tbody>
</table>

a Cutoff values are ≥5% or ≥95% of the controls.
b,c P <0.05. P compared with (21–65 years) group; c compared with (66–80 years) group (ANOVA or χ² test).
c, d d Median (range).
Age correlated directly with the metabolites and inversely with vitamin B12, vitamin B6, and folate. We found a strong correlation between holoTC and vitamin B12 \((r = 0.691; P = 0.001)\). The concentrations of holoTC and creatinine did not significantly correlate \((r = 0.044; P > 0.05)\) (Table 2 in the online Data Supplement).

In line with previous reports \((1–4)\), increased concentrations of the metabolites were more prevalent than decreased concentrations of the vitamin. Only 32% and 20% of individuals >80 years had holoTC ≤ 29 pmol/L or vitamin B₁₂ ≤ 196 pmol/L vs 67% and 39% with increased tHcy and MMA, respectively (Table 1). Free-living elderly individuals were older than those institutionalized, which may explain their lower concentrations of vitamin B₁₂ and folate.

The likelihood of detecting a pathologically increased tHcy or MMA value increased, and that of a low holoTC decreased when renal function decreased. Therefore, increased MMA and holoTC within the reference intervals in individuals with decreasing renal function might not exclude vitamin B₁₂ deficiency. Accordingly, elderly individuals who had higher MMA had also lower holoTC (Fig. 1 in the online Data Supplement).

Vitamin B₁₂ homeostasis may be controlled by the kidney \((16)\). The median concentration of holoTC was similar between the groups with normal and increased creatinine concentrations (54 vs 57 pmol/L, respectively; \(P = 0.129\)). The concentration of holoTC was not significantly lower in individuals of advanced age, which was not explainable in the current study (Table 1). Possible reasons include reduced holoTC filtration by the kidney and/or its cellular uptake.

Individuals who had increased creatinine had also increased concentrations of tHcy, even in the high-normal range of serum folate (Fig. 1C). We therefore anticipate that elderly individuals, particularly those with decreasing renal function, may require higher circulating concentrations of the B vitamins to maintain a normal cellular vitamin status, as might be indicated by serum concentrations of MMA and tHcy.

The holoTC concentration was < 37 pmol/L in all elderly individuals who had tHcy > 19.0 μmol/L and MMA > 0.45 μmol/L \((17)\). Neither food-cobalamin malabsorption nor a poor intake of the vitamin explained the low vitamin B₁₂ and increased MMA and tHcy in the elderly \((3, 18)\). However, use of synthetic vitamin B₁₂ protects against the deficiency of this micronutrient \((19)\). Increased requirements for folic acid, as judged by tHcy normalization, have been reported in elderly individuals \((20)\), whereas younger adults required lower doses of folic...
acidity to normalize their tHcy (21)). Increased requirements for some micronutrients may result from a general decrease in the metabolism with age (22).

Increased MMA and a normal holoTC were found in the current study as well as in a previous one (Table 3 in the online Data Supplement) (23). In a previous study, holoTC showed a higher sensitivity than vitamin B12 when MMA was used as a marker for B12 deficiency (area under the ROC curve, 0.88 vs 0.84) (24). However, the appropriateness of using a single marker or a combination of these markers in elderly individuals should be investigated further. Increased concentrations of MMA or tHcy in the function (25), but that may not exclude vitamin B12 deficiency.

Taken together, the current study in elderly individuals has shown that pathologically increased concentrations of the metabolites are related to subnormal vitamin status as well as to a decrease in renal function. Elderly individuals may require higher circulating concentrations of the B vitamins to maintain concentrations of MMA and tHcy within the reference intervals.

We thank Axis-Shield for providing the reagents for the holoTC assay and Dr. M. Illirich for efforts in recruiting free-living older adults. No organization has supported this study, and we have no conflict of interest related to the context of this report.

References


DOI: 10.1373/clinchem.2003.021717

Immediate Availability of C-Reactive Protein and Leukocyte Count Data Influenced Physicians’ Decisions to Prescribe Antimicrobial Drugs for New Outpatients with Acute Infections, Yuzuru Takemura,1,2* Hideo Kakoi,2 Haku Ishida,3 Hideki Kure,2 Yuriro Tatsuuchi-Harada,2 Masafumi Sugawara,2 Yuji Inoue,2 Ken Ebisawa,2 and Morimitsu Kure2 (1 Department of Laboratory Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan; 2 Department of Internal Medicine, Nishi-Ohmiya Hospital, Saitama-City, Saitama, Japan; 3 Department of Information Technology and Decision Sciences, Yamaguchi University, Ube, Yamaguchi, Japan; * address correspondence to this author at: Department of Laboratory Medicine, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan. Fax 81-42-995-0633, e-mail yutakemu@interlink.or.jp)

Physicians often prescribe antibiotics to febrile patients despite a lack of evidence of bacterial infection (1–5). C-Reactive protein (CRP) and leukocyte count [white blood cell count (WBC)] can contribute to differentiation of possible bacterial vs viral infections (6) because patients with acute bacterial infections typically have markedly increased CRP and WBC whereas patients with viral infection do not. Other studies (7–9) have also suggested...