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Evaluation of Indicators of Cobalamin Deficiency Defined as Cobalamin-induced Reduction in Increased Serum Methylmalonic Acid

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Background: **Early detection of cobalamin deficiency is clinically important, and there is evidence that such deficiency occurs more frequently than previously anticipated. However, serum cobalamin and other commonly used tests have limited ability to diagnose a deficiency state.**

Methods: **We investigated the ability of hematological variables, serum cobalamin, plasma total homocysteine (tHcy), serum and erythrocyte folate, gastroscopy, age, and gender to predict cobalamin deficiency. Patients (n = 196; age range, 17–87 years) who had been referred from general practice for determination of serum cobalamin were studied. Cobalamin deficiency was defined as serum methylmalonic acid (MMA) >0.26 μmol/L with at least 50% reduction after cobalamin supplementation. ROC and logistic regression analyses were used.**

Results: **Serum cobalamin and tHcy were the best predictors, with areas under the ROC curve (SE) of 0.810 (0.034) and 0.768 (0.037), respectively, but age, intrinsic factor antibodies, and gastroscopy gave additional information.**

Conclusions: **When cobalamin deficiency is suspected in general practice, serum cobalamin should be the first diagnostic test, and the result should be interpreted in relation to the age of the patient. When a definite diagnosis cannot be reached, MMA and tHcy determination will provide additional discriminative information, but MMA, being more specific, is preferable for assessment of cobalamin status.**

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Vitamin B_{12} (cobalamin) deficiency can lead to megaloblastic anemia and may cause neuropsychiatric symptoms. Approximately 40% of patients develop only neuropsychiatric symptoms without classical hematological signs *(1, 2)*. When discovered early, these symptoms may be reversed by cobalamin supplementation *(3, 4)*.

Evidence is accumulating that cobalamin deficiency occurs more frequently than previously anticipated, and prevalences of 5–15% in elderly populations have been reported *(5–7)*. Thus, early detection of impaired cobalamin status is of clinical importance. However, determination of serum cobalamin as a test of cobalamin deficiency has certain limitations. Nearly one-half of patients with subnormal serum cobalamin concentrations do not have evidence of intracellular deficiency, whereas many patients with clinically overt cobalamin deficiency have serum concentrations within the reference interval *(8)*. Therefore, various auxiliary diagnostic strategies have been developed.

Patients with classical pernicious anemia show gastroscopic and biochemical signs of chronic atrophic gastritis, which support the diagnosis of cobalamin deficiency *(9, 10)*. However, in the majority of cases, chronic atrophic gastritis is not the result of an autoimmune disease, but is caused by a *Helicobacter pylori* infection, which has a more uncertain relation to cobalamin deficiency *(11–13)*.

Hematologists may base a diagnosis on characteristic changes in the whole blood smear and bone marrow *(9)*, but the diagnostic precision of these morphological variables is limited, and patients without hematological manifestations will be missed by this approach *(1, 14)*.

The imperfection of the classical diagnostic strategies has motivated the development of more reliable tests of

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functional cobalamin status, including plasma total homocysteine (tHcy)[6] and serum methylmalonic acid (MMA). Cobalamin serves as a cofactor in the enzymatic conversion of methylmalonyl CoA to succinyl CoA, and the synthesis of methionine from homocysteine. Impaired intracellular cobalamin status leads to the accumulation of MMA and tHcy in the blood *(15–18)*.

There are few known causes of increased serum MMA other than cobalamin deficiency. Apart from impaired kidney function *(19)*, states of dehydration, and inherited methylmalonic aciduria *(20)*, the only other cause is small-bowel overgrowth with bacteria producing high amounts of propionic acid, the precursor of MMA *(21, 22)*. tHcy, on the other hand, is increased in folate deficiency, vitamin B_6 deficiency, renal failure, proliferative disorders, in response to certain drugs, and in some inborn errors of metabolism *(23, 24)*.

In this study, we evaluated traditional clinical and laboratory tests for diagnosing cobalamin deficiency, using reduction of increased serum MMA after cobalamin supplementation as the gold standard of deficiency. Our aim was to establish a better decision strategy for the diagnosis of this common clinical condition in general practice.

Materials and Methods

SUBJECTS

Subjects were referred to the Department of Clinical Chemistry, Vest-Agder Central Hospital, Kristiansand, Norway by general practitioners for determination of serum cobalamin during the period June 1994 to November 1996. Subjects 16–90 years of age with serum cobalamin ≤300 pmol/L were eligible for the study. Patients with known hematological or malignant disease, or heart failure (New York Heart Association classes III and IV), were excluded. A total of 196 subjects, 63 males and 133 females, fulfilled the inclusion criteria.

STUDY PROTOCOL

In all participants we determined hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), platelet count, serum cobalamin, serum and erythrocyte folate, tHcy, MMA, and serum creatinine. In a subgroup consisting of subjects with serum cobalamin ≤120 pmol/L, MMA >0.26 μmol/L, or tHcy above the 95 percentile (age- and sex-adjusted) of the values found in The Hordaland Homocysteine Study *(25)*, additional investigations were carried out, which included a peripheral blood smear, bone marrow aspiration, gastroscopy with biopsies from the corpus and antrum ventriculi, and determination of intrinsic factor antibodies.

Cobalamin deficiency was defined by initial MMA values >0.26 μmol/L (the upper reference limit), which subsequently decreased by at least 50% 4 weeks after the start of cobalamin injections (1 mg of cyanocobalamin intramuscularly twice weekly for 2.5 weeks). According to this definition, 51 persons had cobalamin deficiency. Subjects with MMA ≤0.26 μmol/L (n = 129) or subjects with MMA >0.26 μmol/L who did not respond to cobalamin treatment by at least a 50% reduction (n = 7) were considered nondeficient. Six subjects with MMA >0.26 μmol/L who for various reasons did not receive cobalamin injections, and three subjects with missing data on MMA were excluded from the statistical analyses.

The study protocol was in accordance with the Helsinki II declaration, and was approved by the regional ethics committee.

LABORATORY METHODS

Serum cobalamin and serum and erythrocyte folate were determined by Bio-Rad Quantaphase II Radioassay (June 1994 to March 1995) or Abbott IMx System (from March 1995). When we compared the two methods as described by Hollis *(26)*, we found no significant bias for serum cobalamin values ≤300 pmol/L [n = 17; 95% confidence interval for the mean difference (Abbott − Bio-Rad) was 0–15 pmol/L]. For folate, the Abbott method gave, on average, results 6% higher than the Bio-Rad method.

Hematological variables were determined by a Cobas Argos analyzer, whereas creatinine was measured by the Vitros System 950. Intrinsic factor antibodies were determined by a ^{57}Co radioassay. Serum MMA *(27)* and plasma tHcy *(28)* were determined by published methods.

Bone marrow aspirate was obtained by sternal puncture and stained with May-Grünwald/Giemsa. Peripheral blood smears were stained with polychrome methylene blue and eosin. Blood and bone marrow smears were investigated by two experienced clinical hematologists blinded to each other, and without knowledge of laboratory data from the patients. The results of blood and bone marrow smears were categorized as normal (0), possibly megaloblastic (1), and megaloblastic (2).

Gastroscopy was performed with an Olympic Videoscope after acid stimulation with 6 μg/kg pentagastrin. On the basis of visual inspection, the results were divided into four categories: normal (0), superficial gastritis (1), atrophic gastritis localized to fundus (2), and generalized atrophic gastritis (3). Gastric acidity after pentagastrin stimulation was used either as a continuous variable (pH) or divided into three categories: achlorhydria (0), pH ≥3 (1), and pH <3 (2). Biopsies were taken from the corpus and antrum ventriculi and were stained with hematoxylin-eosin and investigated at the Department of Pathology, Vest-Agder Central Hospital, Kristiansand, Norway. In addition, the biopsies were stained with combined Alcian-Blue-PAS stain. The results were grouped into

[6] Nonstandard abbreviations: tHcy, total homocysteine; MMA, methylmalonic acid; MCH, mean corpuscular hemoglobin; and MCV, mean corpuscular volume.

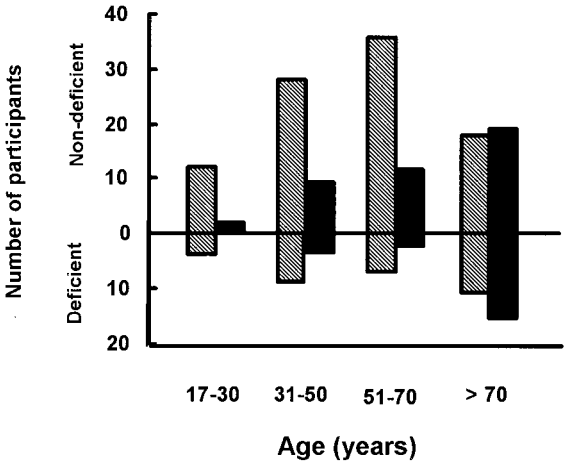

Fig. 1. Age and gender in participants with and without cobalamin deficiency.

▧, females; ■, males.

three categories: normal (0), superficial (not atrophic) gastritis (1), or atrophic gastritis (2).

STATISTICAL ANALYSES

The ability of continuous and categorical variables to predict cobalamin deficiency was evaluated by ROC analysis, using the Astute Statistics Add-in for Microsoft Excel *(29–32)*. To investigate the ability of combinations of variables to predict cobalamin deficiency, logistic regression analysis was performed *(32, 33)*, using SPSS for Windows, Ver. 9.0 (SPSS).

Results

Of the 187 persons included in the statistical analyses, 125 were females and 62 were males. The median age was 59 years, with an age range of 17–87 years. The distribution of age and gender in subjects with and without cobalamin deficiency is shown in Fig. 1.

The distribution of blood test results is shown in Table 1. In nondeficient subjects, serum cobalamin was 51–300 pmol/L, with a median value of 187 pmol/L. In comparison, deficient subjects had overlapping serum cobalamin (range, 24–260 pmol/L) but with a lower median value of 105 pmol/L (Table 1).

None of the cobalamin-deficient subjects had serum creatinine >114 μmol/L. Five nondeficient subjects had serum creatinine concentrations of 115–132 μmol/L and MMA values of 0.07–0.25 μmol/L. The mean creatinine value in the deficient patients was not different from that in the nondeficient group (Table 1). We found no correlation between serum creatinine and MMA values in any of the groups.

ROC ANALYSES

For tests that were performed in all participants, the results of ROC analysis are summarized in Table 2. The ROC curve for the sensitivity-specificity pairs of serum cobalamin is shown in Fig. 2. A large area under the ROC curve suggests good discriminatory power of the variable. If the area under the ROC curve is not significantly >0.5, the variable in question does not distinguish between deficient and nondeficient subjects. Only the first five variables listed in Table 2 had areas under the ROC curves significantly >0.5, and among these, serum cobalamin and tHcy were the best discriminators. Age, MCV, and MCH afforded less discrimination. No significant discriminatory power was provided by serum or erythrocyte folate, hematocrit, or hemoglobin.

When the decision threshold for tHcy was set to 15.0 μmol/L, the sensitivity was 0.73 and the specificity was 0.68. In comparison, the same sensitivity was obtained by serum cobalamin at a threshold of 116 pmol/L, and the corresponding specificity was slightly higher, at 0.74. Thus, these two tests provided essentially the same discrimination at these values. At the commonly used cutoff value for serum cobalamin of 150 pmol/L, the sensitivity was 0.90 and the specificity was 0.60. The same sensitivity

Table 1. Distribution of blood test results in participants with and without cobalamin deficiency.

		Nondeficient (n = 136)				Deficient (n = 51)			
Variable	Units	Median	Range	Mean	SD	Median	Range	Mean	SD
Cobalamin	pmol/L	187	51–300	181	69	105	24–260	108	44
Hemoglobin	g/L	133	93–165	134	13	131	67–163	130	19
Hematocrit		0.40	0.26–0.49	0.40	0.04	0.39	0.19–0.48	0.39	0.06
MCV	fL	93	70–109	93.4	6.4	97	83–127	97.5	9.8
MCH	pg	31	22–38	31.3	2.4	32	27–45	32.7	3.6
Creatinine	μmol/L	76	17–132	78	17	74	40–114	76	15
Serum folate	nmol/L	10.1	4.1–30.6	11.4	5.0	11.8	5.4–29.9	13.2	6.3
Erythrocyte folate	nmol/L	466	110–1056	492	179	420	215–1362	459	186
tHcy	μmol/L	12.9	6.0–91.5	14.8	9.1	19.1	8.8–106.9	25.8	19.9
tHcy[a]	μmol/L					10.7	5.3–81.1	13.4	11.3
MMA	μmol/L	0.16	0.01–0.35	0.16	0.07	0.53	0.27–8.03	0.88	1.23
MMA[a]	μmol/L					0.12	0.03–0.89	0.14	0.12

[a] After cobalamin supplementation.

Table 2. ROC analysis of predictors of cobalamin deficiency.

Variable[a]	Area under the ROC curve	SE
Cobalamin	0.810	0.034
tHcy	0.768	0.037
Age	0.620	0.050
MCV	0.609	0.048
MCH	0.599	0.049
Serum folate[b]	0.587	0.047
Erthrocyte folate[c]	0.574	0.046
Hematocrit	0.564	0.052
Hemoglobin	0.560	0.050

[a] The variables were sorted by the area under the ROC curve. For variables above the horizontal line, the area under the ROC curve was significantly >0.5 (P <0.05).

[b] Higher serum folate values indicate a more positive test result.

[c] Lower erythrocyte folate values indicate a more positive test result.

was obtained for tHcy at a threshold of 11.3 μmol/L, and the corresponding specificity was only 0.38.

Increased tHcy can also be caused by folate deficiency *(23)*. Of 81 patients with tHcy >15.0 μmol/L, 37 had cobalamin deficiency and the remaining 44 had erythrocyte folate concentrations of 127–829 nmol/L (median, 373 nmol/L; mean, 413 nmol/L; SD, 152 nmol/L). Of these, only seven patients had erythrocyte folate <300 nmol/L, of which two were <150 nmol/L. Thus, factors other than cobalamin or folate deficiency appeared to be responsible for increased tHcy in some subjects.

LOGISTIC REGRESSION ANALYSES

To study the diagnostic accuracy provided by combinations of tests, logistic regression analysis *(32, 33)* was performed. We included cobalamin, tHcy, age, hemoglobin, MCV, MCH, hematocrit, erythrocyte and serum folate, and gender in a forward likelihood ratio stepwise procedure. In this model, serum cobalamin was the best predictor of cobalamin deficiency. With serum cobalamin in the model, only age and tHcy gave significant additional information (i.e., regression coefficients were significantly different from zero, P <0.05; Table 3).

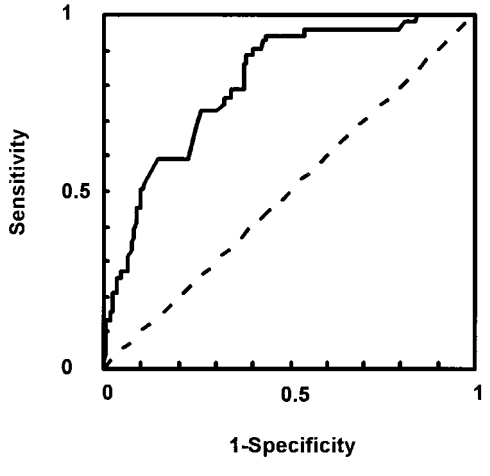

Fig. 2. ROC curve for serum cobalamin as predictor of cobalamin deficiency.

The *dashed line* indicates a test with no predictive power (where the area under the curve = 0.5).

Table 3. Logistic regression analysis of predictors of cobalamin deficiency.

Variable[a]	Regression coefficient	SE	*P*
Cobalamin	−0.0246	0.0052	<0.001
Age	0.0266	0.0116	0.022
tHcy	0.0312	0.0157	0.047
Constant	0.1115	0.9196	

[a] In addition to cobalamin, tHcy, and age, hemoglobin, MCV, MCH, hematocrit, erythrocyte and serum folate, and gender were evaluated. Only variables with regression coefficients significantly different from zero (P <0.05) were included in the final model.

The correlation between serum cobalamin and probability of cobalamin deficiency at ages 20–80 years (in a model not including tHcy) is shown in Fig. 3A. At the age of 20 years, a serum cobalamin value <47 pmol/L was associated with a >50% probability of cobalamin deficiency. At 40, 60, and 80 years, the cobalamin concentrations associated with a >50% probability of cobalamin deficiency were <72, <97, and <120 pmol/L, respectively.

The correlation between serum cobalamin and probability of cobalamin deficiency at various tHcy concentrations at age 60 years is illustrated in Fig. 3B. Notably, the probability of cobalamin deficiency was positively related to tHcy over a wide range of cobalamin concentrations.

ROC analyses were performed using the logistic result as the variable, with and without tHcy in the model. The areas under the ROC curves were as follows (SE in parentheses): with cobalamin, age, and tHcy in the model, 0.836 (0.033); with only cobalamin and age in the model, 0.825 (0.034).

EVALUATION OF DIAGNOSTIC PROCEDURES CARRIED OUT IN SUBJECTS WITH LOW SERUM COBALAMIN, HIGH MMA, OR HIGH tHcy

In a subgroup (n = 108) of the included patients, selected on the basis of serum cobalamin ≤120 pmol/L, MMA >0.26 μmol/L, or tHcy above the 95 percentile of a reference population *(25)*, invasive procedures such as bone marrow aspiration and gastroscopy with biopsies were performed. In addition, peripheral blood smears were performed in these patients. The results of ROC analysis of these data are shown in Table 4.

Low gastric acidity and atrophic gastritis seen at gastroscopy or in gastric biopsies were significant predictors of cobalamin deficiency (Table 4). However, logistic regression analysis showed that when any one of these three variables was included in the model, the other two did not give significant additional information. A close

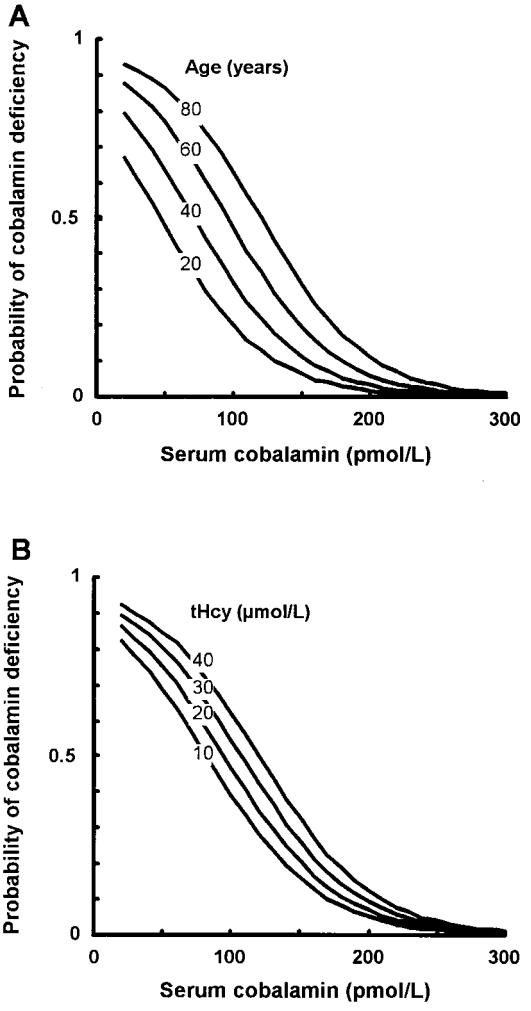

Fig. 3. The probability of cobalamin deficiency according to serum cobalamin, age, and tHcy.

The probability was obtained by logistic regression analysis. (*A*), the probability of cobalamin deficiency according to serum cobalamin and age, without tHcy in the model. The regression coefficients (SE in parentheses) were as follows: cobalamin, −0.0261 (0.0051); age, 0.0319 (0.0114); constant, 0.5852 (0.8743). (*B*), the probability of cobalamin deficiency according to serum cobalamin and tHcy. The regression coefficients from Table 3 were used, with the age of the patients set to 60 years.

correlation between the results of these investigations may account for this observation.

Among patients with achlorhydria (n = 16), 69% had cobalamin deficiency. For the remaining patients, there was a positive relationship between cobalamin deficiency and gastric pH over the pH range 0.6–8.2. The logistic regression coefficients (SE in parentheses) were as follows: pH, 0.2451 (0.0935); constant, −1.039 (0.3529).

Peripheral blood smears or bone marrow aspirates had no significant discriminatory power (Table 4). The kappa values for interobserver agreement between the two hematologists were 0.37 for peripheral blood smear and 0.12 for bone marrow aspirate.

Intrinsic factor antibodies were determined in patients who had invasive procedures. The sensitivity and specificity to detect cobalamin deficiency were 0.17 (95% confidence interval, 0.06–0.28) and 0.96 (95% confidence interval, 0.92–0.999), respectively. When patients were subgrouped according to high and low hemoglobin values, the sensitivity and specificity were about the same in both groups.

Table 4. Ability of tests performed in selected patients[a] to predict cobalamin deficiency, as studied by ROC analysis.

Variable[b]	Area under the ROC curve	SE	n
Gastric acidity	0.676	0.052	110
Gastric biopsy	0.666	0.053	107
Gastroscopy (visual)	0.660	0.054	105
Bone marrow smear (A)[c]	0.558	0.061	92
Blood smear (B)	0.523	0.096	38
Bone marrow smear (B)	0.508	0.088	44
Blood smear (A)	0.507	0.066	79

[a] Patients were selected on the basis of serum cobalamin ≤120 pmol/L, MMA >0.26 μmol/L, or tHcy above the 95th percentile of a reference population.

[b] All variables were defined as categories (see text) and sorted by the magnitude of the area under the ROC curve. For variables above the horizontal line, the area under the ROC curve was significantly >0.5.

[c] The letters A and B refer to the hematologists who evaluated the blood and bone marrow smears.

Discussion

Increased serum MMA that responds to cobalamin supplementation with at least 50% reduction provides strong evidence for intracellular cobalamin deficiency *(1, 10, 16, 34)*. When renal insufficiency and inherited disorders of methylmalonic aciduria are excluded, cobalamin deficiency is the predominant cause of increased serum MMA. In a few cases, small-bowel bacterial overgrowth may produce cobalamin malabsorption *(15)*.

When we used a reduction in increased MMA after cobalamin treatment as the gold standard, ROC analyses demonstrated that serum cobalamin and plasma tHcy were the best noninvasive predictors of cobalamin deficiency (Table 2). These two variables provided essentially the same discrimination. Because impaired folate status causes increased tHcy *(35)*, tHcy may have low diagnostic specificity in an unselected population. In addition, there are other causes of increased tHcy *(23, 24)*.

Cobalamin deficiency is associated with increased serum folate, possibly because of failure to take up or retain methyltetrahydrofolate in cells ("folate trapping") *(36, 37)*. However, the serum folate increase that we observed was not sufficient to be used as a diagnostic tool for detection of cobalamin deficiency (Table 2).

ROC analysis gives information about only one test at a time. If results from two tests are correlated, the discriminatory information provided by combining both tests is less than the sum of information from each separate test. In such cases, multivariate logistic regression analysis can be used to determine the additional discriminatory information given by each test in successive diagnostic procedures *(32, 33)*.

Beyond the serum cobalamin concentration, the age of the patient provided additional predictive information (Fig. 3A). In addition, inclusion of tHcy increased the predictive power (Table 3 and Fig. 3B). However, when ROC analysis was done with the logistic result as the variable, the area under the curve was only slightly greater for the combinations of tests than for cobalamin alone (see *Results*).

Intrinsic factor antibodies (determined only in the group selected on the basis of low serum cobalamin, high tHcy, or high MMA) achieved very low sensitivity (0.17) but high specificity (0.96). Obviously, many of our patients were cobalamin-deficient from causes other than classic pernicious anemia.

No other blood test provided significant additional information; the only exception was serum MMA, which we used as the gold standard to define cobalamin deficiency. In other words, our data suggest that the only blood tests that add significant diagnostic information beyond that provided by serum cobalamin are tHcy, MMA, and if positive, intrinsic factor antibodies. The erythrocyte indices MCV and MCH may be of value when used alone (Table 2), but they add no predictive power beyond serum cobalamin (Table 3).

Evaluation of blood and bone marrow smears had no predictive value in our study (Table 4). This finding was corroborated by the poor interobserver agreement between the two hematologists. Changes in peripheral blood and bone marrow probably do not become evident before late stages of cobalamin deficiency, and the majority of our patients were probably in an early phase of the disease. One may object that these diagnostic procedures were performed in selected patients. However, a similar selection is done in clinical practice because these tests are usually preceded by less time- and resource-demanding tests.

The presence of atrophic gastritis, a high pH in the stomach aspirate, and achlorhydria were highly correlated phenomena. Any one of these observations significantly increased the probability of cobalamin deficiency even after serum cobalamin and/or plasma tHcy had been determined (Table 4).

Seven patients with MMA above our cutoff of 0.26 μmol/L had a <50% reduction in MMA after cobalamin supplementation and were therefore classified as nondeficient. That means that using MMA >0.26 μmol/L (without cobalamin supplementation) as a diagnostic test for cobalamin deficiency would have a sensitivity of 1.00 and a specificity of 0.95 according to our criteria. Because 0.26 μmol/L is our reference limit for MMA, 2.5% of healthy individuals can be expected to have higher values, and the expected specificity of this approach is thus 0.975.

It cannot be ruled out that some cobalamin-deficient patients have MMA ≤0.26 μmol/L or fail to show a 50% reduction in MMA after cobalamin supplementation. These patients are not recognized by our approach. The number of such false negatives is not known. On the basis of other criteria, Lindenbaum et al. *(15)* suggested a sensitivity for MMA of 0.95, but they used a higher cutoff value.

In a recent report, Hølleland et al. *(38)* defined cobalamin deficiency by serum MMA >0.376 μmol/L, which is 3 SD above the mean of healthy controls. In our study, 13 patients who were cobalamin-deficient according to our criteria had serum MMA between 0.26 and 0.376 μmol/L, whereas all patients with MMA >0.376 μmol/L had a serum MMA reduction ≥50%. Thus, in our study population, use of a cutoff for serum MMA of >0.376 μmol/L (without cobalamin supplementation) affords diagnostic sensitivity and specificity of 0.75 and 1.00, respectively.

On the basis of a cost-benefit analysis, Hølleland et al. *(38)* recommended measurement of MMA in patients when serum cobalamin is >60–90 pmol/L and <200–220 pmol/L. A similar recommendation was made in a recent review *(39)*. Other algorithms also emphasize that cobalamin deficiency should not be ruled out on the basis of normal serum cobalamin values alone *(40)*.

In this study, we emphasized the importance of taking a patient's age into consideration. From the calculations presented here, we conclude that in the individual patient a definite diagnosis of cobalamin deficiency can be based with reasonable certainty on serum cobalamin only when a very low cobalamin value is found in a patient of advanced age (Fig. 3A). On the other hand, exclusion of cobalamin deficiency seems justified in subjects with high cobalamin values, especially in young people, although the results shown in Fig. 3 should be interpreted with caution. In other cases, the definite diagnosis or exclusion of cobalamin deficiency should not be based on serum cobalamin alone, but auxiliary tests such as measurement of MMA or tHcy should be performed.

The patients in this study were referred from their primary physicians for determination of serum cobalamin. Thus, the above decision strategy is relevant for the diagnosis of cobalamin deficiency in general practice. It may be prudent to determine folate status as well, because concomitant folate deficiency may be present.

The main limitation to a more widespread use of MMA as the primary test is its high cost. The costs of MMA analysis are ~10-fold higher than that of serum cobalamin. Until more inexpensive methods for MMA determination are developed, serum cobalamin may keep its position as the first-line diagnostic test of cobalamin deficiency.

In conclusion, the results of the present study of subjects with suspected cobalamin deficiency in general practice support the use of serum cobalamin as a first-line diagnostic procedure. Low serum cobalamin values should be interpreted in relation to the age of the patient because the probability of a deficiency state increases with increasing age. In those cases where a likely diagnosis cannot be reached, determination of serum MMA and tHcy may provide additional discriminative information, but MMA is preferable because it combines high sensitivity and specificity. Blood and bone marrow morphologies have poor discriminative power, whereas invasive procedures such as

gastroscopy, gastric biopsy, and determination of gastric acidity provide both diagnostic and causal information.

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