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Test Utilization and Outcomes

Cobalamin Deficiency in General Practice. Assessment of the Diagnostic Utility and Cost-Benefit Analysis of Methylmalonic Acid Determination in Relation to Current Diagnostic Strategies

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Diagnosing cobalamin deficiency is often difficult. We investigated the diagnostic strategies that 224 general practitioners used to assess cobalamin status and the criteria on which they based their decisions to supplement patients. From all serum cobalamin analyses carried out at a single laboratory during 1993, individuals with serum cobalamin concentrations <300 pmol/L were identified, and one patient per general practitioner was included. When serum methylmalonic acid (s-MMA) values >0.376 μmol/L were used as the "reference standard" for cobalamin deficiency, the serum cobalamin assay had a diagnostic sensitivity of 0.40 and a specificity of 0.98. With the same reference standard, the diagnostic accuracy of the physicians' decision to supplement patients had the same specificity but a higher sensitivity (0.51). Cost-benefit analysis indicated that measurement of s-MMA can be recommended in patients with serum cobalamin >60–90 pmol/L and <200–220 pmol/L, depending on its diagnostic accuracy.

The diagnosis of cobalamin deficiency is traditionally based on medical history and the finding of megaloblastic anemia combined with a low serum cobalamin (s-cobalamin)[4] concentration. Development of cobalamin deficiency is usually a slow process that often starts with subtle symptoms such as asthenia, memory impairment, or polyneuropathy *(1–6)*. Moreover, severe neurologic deficits may occur in the absence of anemia in a considerable portion of patients *(7)*. The often diffuse and nonspecific symptoms in the early phase of cobalamin deficiency, the possible absence of anemia, and the irreversible damage to the central nervous system that may result from delayed diagnosis *(8, 9)* are strong incentives to establish accurate diagnostic tests. A poor diagnostic test for cobalamin deficiency can also lead to overdiagnosis, which may in turn lead to people being treated their entire lives for a disease they do not have and which may also mask other diseases.

Among laboratory tests for the diagnosis of cobalamin deficiency, determination of s-cobalamin is most commonly used. However, the diagnostic efficiency of s-cobalamin is too low . This can partly be explained by the fact that the major fraction of cobalamin in serum is bound to haptocorrin, which is not available for uptake in most cells *(10, 11)*. Approximately 20% is bound to transcobalamin II. Only the transcobalamin II-cobalamin complex can be utilized by peripheral cells through receptor-mediated uptake *(12, 13)*. In addition, s-cobalamin may be influenced by changes in the binding protein concentration. Thus, total s-cobalamin is a relatively poor indicator of bioavailable cobalamin *(14)*. Therefore, tests for other cobalamin markers have been developed, such

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[4] Nonstandard abbreviations: s, serum; MMA, methylmalonic acid; tHcy, total homocysteine; FML, Fürst Medical Laboratory; and CI, confidence interval.

as the holo-transcobalamin II concentration in serum *(15)*, the deoxyuridine suppression test, or the functional markers methylmalonic acid (MMA) and total homocysteine (tHcy) *(16)*.

Among the functional tests, determination of serum MMA (s-MMA) has received particular attention. This is because of the stability of MMA in blood *(17)* and the small sample volume requirements and high diagnostic accuracy of the test. s-MMA is assumed to be a better indicator of intracellular cobalamin status than s-cobalamin, and s-MMA has been proposed as a complement or replacement for s-cobalamin measurements *(17)*. However, methods for MMA measurement are costly and cumbersome, and commercial assays are not available. This hampers its widespread routine use.

Here, we report on the diagnostic strategies used to assess cobalamin deficiency in general practice, especially the influence of s-cobalamin assay results on final diagnosis and the decision to supplement patients. We also estimated the increase in diagnostic accuracy obtained by additional determination of s-MMA, and indicate the s-cobalamin range, where this may be justified, based on cost-benefit analyses.

Patients and Methods

PATIENTS AND STUDY DESIGN

The study was performed as a retrospective cohort study. Patients were recruited through the Fürst Medical Laboratory (FML) in Oslo, Norway in 1993. During that year, a total of 76 840 cobalamin analyses were performed, and only patients with s-cobalamin concentrations <300 pmol/L (30%) were eligible for the present study. The lower reference limit for s-cobalamin was 170 pmol/L. Approximately 75 patients in each of the following s-cobalamin concentrations were included: 0–139, 140–169, 170–189, 190–219, and 220–299 pmol/L. The limits of the intervals were chosen in this way to ensure that there were enough patients representing definitely low, borderline, and health-related values of s-cobalamin because we were especially interested in the physicians' decision when they received results in these concentrations. Only one patient per general practitioner was included.

Approximately 6 weeks after FML had sent the results of cobalamin analysis, the general practitioners received a questionnaire to obtain information on why they had requested s-cobalamin measurement. The categories provided in the questionnaire were anemia; macrocytosis; psychiatric symptoms (specified as depression, dementia, or psychosis); neurologic symptoms (unspecified); and diffuse symptoms, screening, and others. Other questions asked whether additional analyses were requested, whether the general practitioner believed that the patient was cobalamin deficient, and whether the patient had received any treatment. General practitioners who did not respond to the first questionnaire received a new questionnaire after 6 weeks.

A total of 316 (84%) out of 376 questionnaires were returned. Five were excluded from all further analyses because the doctors were not general practitioners. Nine patients were excluded because the questionnaires were incompletely filled out. In addition, we did not ask for clinical data in 78 cases where s-cobalamin was ordered as a confirmation test of either an earlier s-cobalamin determination or as therapy control in patients on cobalamin supplementation. Thus, 224 patients were qualified for additional data analysis. In addition, 15 patients had to be excluded from parts of the study because there was not enough serum left for measurement of serum concentrations of creatinine and MMA. Likewise, 12 women and 7 men with serum creatinine >115 and 125 μmol/L, respectively, were excluded from parts of the study where s-MMA was used *(18–20)*. Table 1 shows the demographic characteristics of the study population.

Table 1. Demographic representation of the study population as percentages of the total number of samples included.

	s-Cobalamin interval, pmol/L				
	0–139	**140–169**	**170–189**	**190–219**	**220–299**
Total number of cobalamin determinations at FML in 1993 (n = 76 840)[a]					
Percentages in each interval	1.0	1.8	2.0	4.9	20.1
Age in years, median (range)	62 (1–93)	68 (16–68)	66 (9–96)	62 (13–98)	58 (1–97)
Female/male ratio	1.3	1.8	1.9	2.1	1.9
Included in the study (n = 376)					
Percentages in each interval	20.1	22.0	18.7	19.2	20.1
Age in years, median[b]	76	74	65	64	57
Female/male ratio	1.9	1.9	2.4	1.9	1.9
Included in the statistical analyses (n = 224)					
Percentages in each interval	22.0	16.6	18.7	19.2	20.1
Age in years, median[b]	69	68	59	61	57
Female/male ratio	1.3	1.6	2.9	1.5	1.9

[a] 22 892 samples with cobalamin values <300 pmol/L (29.8%) were included.
[b] Range, 18–90 years.

SAMPLE HANDLING AND BIOCHEMICAL ANALYSES

In this study, the blood was collected and the serum prepared at the general practitioners' offices. The serum samples were then mailed to FML. After FML had performed the requested blood analyses, the serum samples were stored at −20 °C. s-Creatinine, s-tHcy, and s-folate were later measured at the Laboratory of Clinical Biochemistry, Haukeland Hospital, Bergen, Norway, whereas s-MMA was analyzed at the Department of Pharmacology, University of Bergen.

s-Cobalamin and s-folate were measured by RIA (Diagnostic Product Corp.). The reference range of s-cobalamin was 170–700 pmol/L, and the between-day CV was 3–7%, depending on the s-cobalamin concentration. The reference limit of s-folate was >5.0 nmol/L, and the between-day CV was 3–7%, depending on the s-folate concentration.

Serum creatinine was measured by the Jaffe method on an Axon® (Bayer Instruments Corp.). The reference ranges for serum creatinine were 55–115 and 60–125 μmol/L for women and men, respectively. The between-day CV was 2.5–3.5%. In 15 patients, there was not sufficient serum for creatinine determination, and these were excluded from part of the analyses.

Serum tHcy, which includes free and protein-bound Hcy forms, was determined by a modification of an automated procedure based on derivatization with monobromobimane, followed by HPLC and fluorescence detection *(21, 22)*. The between-day CV was ~3%. The reference range for serum tHcy is 0–15 μmol/L, and results above 15.0 μmol/L are considered increased *(23)*.

s-MMA was measured by capillary electrophoresis *(24)*. The between-day CV was 5–10% for MMA concentrations within a range of 0.12–0.57 μmol/L. The reference range for s-MMA is 0.05–0.26 μmol/L. The cutoff point for diagnosing functional cobalamin deficiency was set to 0.376 μmol/L, which corresponded to 3 SD above the mean of healthy controls *(22, 25–28)*. Lower values were referred to as "normal".

STATISTICS AND DATA ANALYSES

Data analyses were performed using multivariate stepwise logistic regression analyses (SPSS, Ver. 4.0 for Macintosh). From this we obtain the log odds and could remodel the equation to give the posttest probability of an event. In the logistic regression analyses, different dependent and independent variables were used.

Results and Discussion

Cobalamin deficiency presenting with classical clinical manifestations is not difficult to diagnose. However, such a clinical picture is seen only rarely, even in a selected group of patients with s-cobalamin in the lower range. Conceivably, the indications for requesting such a test will differ with the cobalamin concentration found. Likewise, because there is no independent "gold standard" for cobalamin deficiency, the certainty with which the general practitioner sets a diagnosis, and the probability that the patient will be supplemented, will be directly related to the serum cobalamin assay result. When comparing s-cobalamin with s-MMA, we therefore must consider whether the additional information obtained by measuring MMA will assist the general practitioner in reaching the correct diagnosis and thereby the correct therapy.

In the present study, we investigated the diagnostic strategies among 224 general practitioners, each contributing one patient with a serum cobalamin <300 pmol/L. The patients belonged to five different categories defined according to their cobalamin concentration. This allowed us to investigate the different diagnostic approaches as a function of the cobalamin concentration. We first registered the indications that the general practitioner listed for requesting the serum cobalamin assay. We then investigated how the general practitioner responded to the serum cobalamin assay result as judged by (*a*) the subsequent request of additional biochemical tests; (*b*) their ability to set a definite diagnosis; and (*c*) the initiation of cobalamin therapy.

We performed a retrospective comparison of the s-MMA as the reference standard with the initial serum cobalamin assay result, the ability of the general practitioner to set a definite diagnosis, and the initiation of cobalamin supplementation. On the basis of these results, we were able to evaluate the diagnostic benefit of including MMA as an additional marker of cobalamin deficiency.

INDICATIONS FOR REQUESTING S-COBALAMIN

The indications for requesting s-cobalamin are summarized in Table 2. Two or more indications were frequently reported. Anemia was an indication for requesting s-cobalamin in ~30% of patients with s-cobalamin values <170 pmol/L. This percentage decreased at higher s-cobalamin concentrations. Macrocytosis was an uncommon reason for cobalamin testing, even at low cobalamin concentrations. In almost 50% of the patients with s-cobalamin <190 pmol/L, the general practitioners did not report hematological, neurologic, or psychiatric findings or symptoms.

PRIMARY AND ANCILLARY TESTS

Hemoglobin was requested together with s-cobalamin for 70–90% of the patients. s-Iron, s-total iron binding capacity, or s-ferritin was requested for 60–80%, thyroid function tests were requested for 30–50%, and serum or red cell folate was requested for ~30% of the patients. s-Lactate dehydrogenase, s-bilirubin, or the Schilling test were seldom ordered as initial or ancillary tests (data not shown).

Cobalamin retesting was the most frequently used follow-up test in these patients (Table 3). With s-cobalamin in the range of 170–189 pmol/L, 66% of the general practitioners ordered a new test within 6 weeks. Examination of blood smears, gastroscopy, or measurement of

Table 2. Indications for requesting cobalamin.

	s-Cobalamin interval, pmol/L				
	0–139	140–169	170–189	190–219	220–299
Number of patients	51	37	35	48	52
Indications (% within each cobalamin interval)[a]					
Anemia (A)	31	35	23	15	4
Macrocytosis (M)	8	5	3	0	0
Psychiatric/neurological symptoms (P/N)	31	27	31	27	25
P/N without anemia or macrocytosis	22	19	29	23	35
Screening, diffuse or other symptoms (SDO)	73	78	80	88	35
SDO without A, M, or P/N	43	46	49	63	62

[a] The vertical sums exceed 100% because the categories are not mutually exclusive and there were often two or more indications reported.

s-antibodies against intrinsic factors or parietal cells were only performed in a few patients.

influence of s-cobalamin concentrations on the clinical diagnosis

We investigated whether the physicians had made a definite diagnosis regarding the cobalamin status of their patients. The alternatives given were "deficient", "not deficient", and "uncertain". As shown in Table 4, the category uncertain was the diagnosis for >50% of the patients with s-cobalamin between 140 and 219 pmol/L and 41% of the patients with s-cobalamin <140 μmol/L. In contrast, the majority of general practitioners were able to make a definite diagnosis of not deficient or deficient in patients belonging to the highest and lowest cobalamin intervals. Only 2–9% of the patients with s-cobalamin between 170 and 299 pmol/L were regarded as deficient, and <10% of the patients with s-cobalamin <170 pmol/L were diagnosed as not deficient (Table 4).

In a logistic regression analysis, we used the physician's diagnosis as the response variable (deficient = 1, not deficient = 0) and indications for requesting s-cobalamin (Table 2), s-cobalamin, and the patient's age and sex as independent variables. In this model, s-cobalamin (decreasing values in intervals of 10 pmol/L) and anemia (no = 0; yes = 1) significantly predicted the clinical diagnosis deficient, with odds ratios of 1.34 [95% confidence interval (CI), 1.21–1.48] and 3.95 (95% CI, 1.08–14.5), respectively. Patients classified as uncertain were not included in this model.

evaluation of the diagnostic strategies

By definition, 2.5% of healthy people have values below the lower reference limit of a quantitative laboratory test *(25)*. If there is prior clinical selection of the subjects to be tested, the percentage is expected to be higher. However, we found that only 2.8% of routinely measured s-cobalamin values were <170 pmol/L (Table 1). This may be explained by the indications for ordering s-cobalamin in our survey (Table 2). Patients with anemia and/or macrocytosis have a relatively high pretest probability of cobalamin deficiency *(26, 29–31)*; however, these indications represent a minority in our survey results. An additional explanation might be the use of s-cobalamin as a control test during cobalamin supplementation. Furthermore, in the majority of cases, s-cobalamin was requested for screening purposes or for examination of diffuse symptoms, which is also indicated by the frequent request of other biochemical tests together with s-cobalamin. Thus, s-cobalamin is most likely used as a case-finding test for cobalamin deficiency in general practice *(32)*.

The high portion of subjects categorized as uncertain diagnosis (Table 4) may indicate that general practitioners are aware of the low predictive value of s-cobalamin. However, there could be a bias towards this category because general practitioners may prefer to categorize

Table 3. Follow-up tests for investigating suspected cobalamin deficiency.

	s-Cobalamin interval, pmol/L				
	0–139	140–169	170–189	190–219	220–299
Number of patients	51	37	36	48	52
Follow-up tests (% within each cobalamin interval)					
Cobalamin retesting	22	19	66	27	13
Blood smear	29	19	14	10	15
Mean corpuscular volume	29	8	9	8	4
Folic acid	47	39	37	37	33
Gastroscopy	8	5	6	6	2
Intrinsic factor antibody	17	3	14	14	2
Parietal cell antibody	2	0	0	0	0

Table 4. Relationship between the general practitioner's clinical diagnosis and the cobalamin concentration.

	s-Cobalamin interval, pmol/L				
	0–139	140–169	170–189	190–219	220–299
Number of patients	51	37	36	48	52
General practitioner's clinical diagnosis (% within each cobalamin interval)					
Not deficient	6	8	17	46	65
Uncertain diagnosis	41	54	74	52	33
Deficient	53	38	9	2	2

their patients as uncertain diagnosis to avoid making a false-negative diagnosis. The number of patients with uncertain diagnoses increased around the lower reference limit of s-cobalamin. This observation is corroborated by the frequent cobalamin retesting in patients with low and low-normal values (Table 3). In spite of this considerable diagnostic uncertainty, ancillary tests such as the mean corpuscular volume, a blood smear, or the intrinsic factor antibody are seldom used.

SERUM CONCENTRATIONS OF VITAMINS AND METABOLIC MARKERS

Different reference standards for functional cobalamin deficiency have been presented. The deoxyuridine suppression test (dUST) is theoretically sound *(27, 33)*, but is expensive and labor-intensive, and therefore impractical for large-scale use. Moelby et al. *(32)* used an abnormal Schilling test and/or megaloblastic bone marrow morphology, which could not be explained by folate deficiency, as the reference standard. Increased s-MMA and/or s-tHcy or a substantial decrease in these metabolites after cobalamin injections have been used by other investigators *(19, 28, 34)*.

In 1993, s-MMA was not available as a routine analysis in Norway, and tHcy was little known and not available at FML. The physicians, therefore, made their diagnoses without knowledge of the s-MMA or tHcy values for their patients.

The distributions of increased s-MMA, tHcy, and low s-folate at different s-cobalamin concentrations are shown in Table 5. As expected, tHcy was higher at low s-cobalamin concentrations and decreased as a function of the cobalamin concentration. In contrast, s-folate showed no relationship with s-cobalamin, and <11.5% of the patients had s-folate <5 nmol/L throughout the s-cobalamin strata (Table 5).

The relationship between s-MMA and tHcy is depicted in Fig. 1. tHcy was increased in a substantial number of patients with s-MMA values within the reference range, whereas only six patients with reference values of tHcy (<15 μmol/L) had increased s-MMA (Fig. 1). This may reflect the lower specificity of s-tHcy as an indicator of cobalamin deficiency *(35)*. In accordance with these data and with procedures advocated by others *(19, 20, 34, 36, 37)*, we therefore used s-MMA >0.376 μmol/L (3 SD above the mean of the reference range) as the reference standard in this study.

ASSESSMENT OF DIAGNOSTIC ACCURACY OF S-COBALAMIN

The s-cobalamin concentration was the basis for patient selection in this study. We therefore had to correct for the frequency distribution of s-cobalamin values when calculating the sensitivities and specificities of s-cobalamin. This correction was done on the basis of the frequency distribution of the total s-cobalamin analyses at FML in 1993 (Table 1).

We then evaluated the diagnostic sensitivity and specificity of s-cobalamin ≤170 pmol/L, using s-MMA >0.376 μmol/L as the reference standard. The sensitivity of s-cobalamin was 0.40 (95% CI, 0.22–0.58), and the specificity was 0.98 (95% CI, 0.976–0.983). In these calculations,

Table 5. Concentrations of MMA, t-Hcy, and folate in serum in different cobalamin intervals.

	s-Cobalamin interval, pmol/L					
	0–139	140–169	170–189	190–219	220–299	All
MMA						
Median, μmol/L	0.46	0.28	0.21	0.18	0.15	0.23
Conc.[a] >0.37 μmol/L, %	59.2	32.9	24.6	8.1	4.6	26.7
t-Hcy						
Median, μmol/L	20.1	14.7	13.5	12.0	11.7	13.6
Conc. >15.0 μmol/L, %	70.4	49.3	40.0	25.4	24.6	42.5
Folate						
Median, nmol/L	10.0	9.9	10.1	10.1	10.8	10.2
Conc. <5.0 nmol/L, %	7.8	10.8	4.8	11.5	1.5	7.2

[a] Conc., concentration.

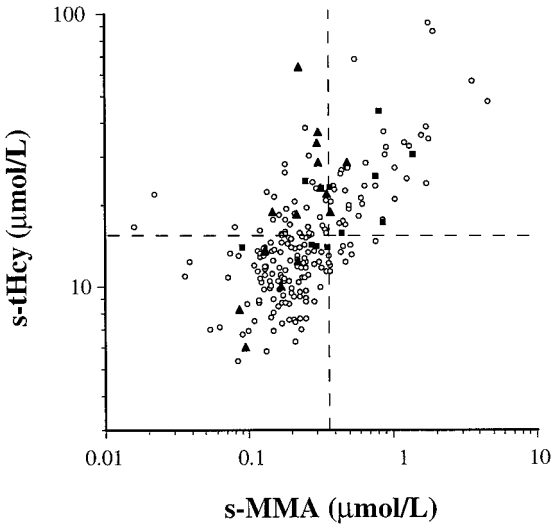

Fig. 1. Correlation between s-tHcy and s-MMA.

■, patients with increased s-creatinine; ▲, patients with low s-folate; ○, patients with s-creatinine and s-folate within the reference range.

we have assumed that there are no cobalamin-deficient patients with s-cobalamin >300 pmol/L.

These results, which showed low sensitivity for s-cobalamin, are in line with other studies. Norman et al. *(38)* discovered that 51% (18 of 35) of elderly people with increased urinary MMA had s-cobalamin below the lower reference limit. Pennypacker et al. *(28)* found that 7 (37%) of 19 geriatric outpatients with s-MMA >0.376 μmol/L had s-cobalamin <148 pmol/L, and Nilsson et al. *(39)* estimated the sensitivity of s-cobalamin <150 pmol/L to be 41% in a psychogeriatric population. In surviving members of the original Framingham elderly population, only 12 (20%) of 59 patients with increased s-MMA and a s-creatinine value within the reference range had s-cobalamin <148 pmol/L *(20)*.

The estimated diagnostic accuracy of a test is always influenced by the selection of the study population. The specificity will usually be lowest in populations with high prevalence of other/similar diseases, and the sensitivity will be highest in populations with advanced disease *(38)*. Accordingly, we would expect our estimate of specificity to be high and our sensitivity to be low compared with a group of inpatients.

The overall prevalence of cobalamin deficiency in the patients having their s-cobalamin tested at FML was estimated to 2.96%, when increased s-MMA (>0.376 μmol/L) was used as the reference.

PREDICTORS OF HIGH S-MMA

In multiple logistic regression analyses, we used indications for requesting s-cobalamin (Table 2), s-cobalamin, s-creatinine, and patient age and sex as independent variables. In this model, increased s-MMA was significantly predicted by low s-cobalamin (decreasing values in intervals of 10 pmol/L), high age, and female sex (coded = 1), with odds ratios of 1.34 (95% CI, 1.22–1.63), 1.12 (95% CI, 1.08–1.17), and 3.12 (95% CI, 1.09–9.1), respectively.

A similar relationship between s-MMA, s-cobalamin, age, and sex was found by Pennypacker et al. *(28)*. Norman et al. *(38)* found increased urinary MMA in 5.3% of women compared with 1.9% of men. They found no statistically significant association between age and urinary MMA concentrations. However, their study population consisted only of persons >65 years of age.

An independent effect of age and sex on the probability of functional cobalamin deficiency at the same concentration of s-cobalamin requires substantiation.

CLINICAL DIAGNOSIS AND THERAPEUTIC CONCLUSION IN RELATION TO CONCENTRATIONS OF MMA AND COBALAMIN

The relationship between the general practitioners' clinical diagnoses and the s-MMA and s-cobalamin concentrations is illustrated in Fig. 2. There is a considerable discrepancy between the general practitioners' diagnosis of "cobalamin-deficient" and the number of patients with increased MMA (Fig. 2A). This is especially true for higher cobalamin concentrations. On the other hand, the agreement between the clinical diagnosis not deficient and s-MMA values within the reference range is very good (Fig. 2C). Only one patient with s-MMA >0.376 μmol/L was diagnosed as not deficient. Nine percent of patients, whom the general practitioners had classified as uncertain, had increased s-MMA (Fig. 2B).

The decision by a general practitioner to start cobalamin supplementation was also made when the diagnosis was uncertain. In this case, low s-cobalamin seemed particularly influential because cobalamin supplementation was given to

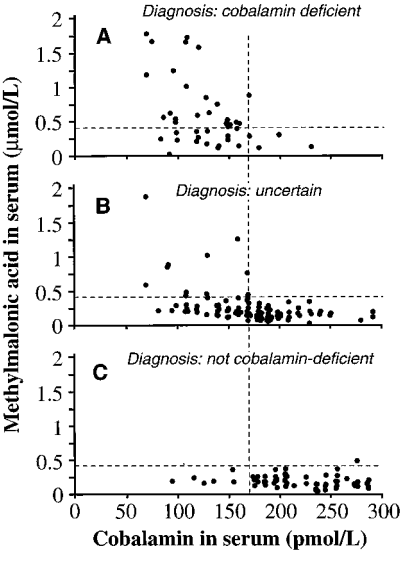

Fig. 2. Relationship between s-MMA and the general practitioners' diagnoses.

Diagnoses: *A*, cobalamin-deficient; *B*, uncertain; *C*, not deficient. Patients with increased s-creatinine have been excluded.

patients in the uncertain category almost exclusively when they had s-cobalamin <170 pmol/L (Table 6).

In the group classified as deficient by the general practitioners, two patients were advised to change their diets, two were followed up by other general practitioners, one refused any treatment, and one died before therapy was started. The rest of the patients received supplementation with cobalamin. For the estimates below, all patients classified as deficient by the general practitioners were included in the group of patients receiving cobalamin supplements. When supplementation was used as the response indicator and given the assumption that no patients with s-cobalamin >300 pmol/L were supplemented, the clinical decision to supplement the patient had a sensitivity of 0.51 (95% CI, 0.32–0.69) and a specificity of 0.98 (95% CI, 0.976–0.983).

To compare the diagnostic efficiency of clinical judgement (supplementation or not) with the s-cobalamin assay, again using s-MMA as the reference standard, we performed a logistic regression analysis and calculated "posttest" probabilities. We first used supplementation as the response variable in an analysis with s-cobalamin as the independent variable (Fig. 3, inset). As expected, as s-cobalamin values decreased, the probability that a patient would receive a supplement gradually increased. We next used high s-MMA as the response variable and then analyzed the following three groups separately: patients receiving cobalamin supplementation, patients not receiving supplementation, and all patients. The probability of true cobalamin deficiency (defined by increased MMA) is shown separately for the three groups in Fig. 3.

The importance of clinical judgement can be estimated by the difference in the probability of cobalamin deficiency in the supplemented and nonsupplemented groups. For example, in patients with a s-cobalamin value of 100 pmol/L, the probability that the patient had a cobalamin deficiency was ~0.59. In the supplemented and nonsupplemented groups, the corresponding numbers were 0.73 and 0.31, respectively (Fig. 3).

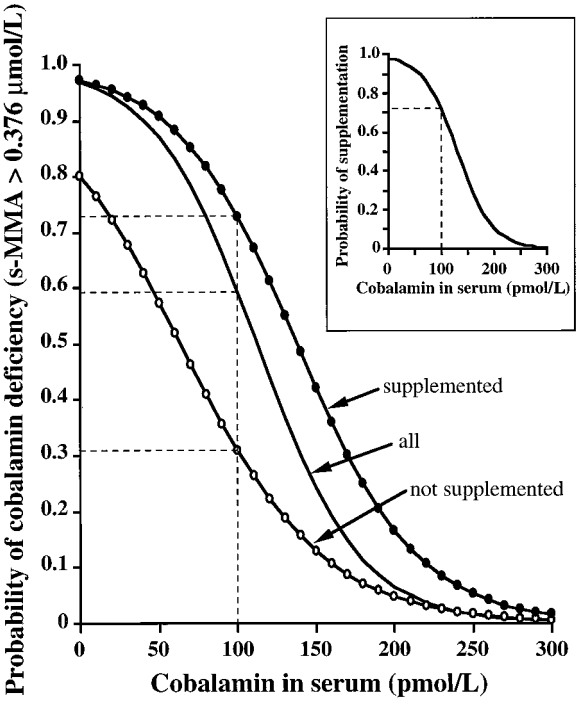

Fig. 3. Relationship between the decision by general practitioners to give cobalamin supplementation and the probability of cobalamin deficiency.

The *inset* shows the probability of cobalamin supplementation at various s-cobalamin concentrations. The *main panel* illustrates the relationship between s-cobalamin and the probability of cobalamin deficiency, using s-MMA as reference standard. Data for supplemented patients (●), all patients (———), and nonsupplemented patients (○) are shown.

DIAGNOSTIC BENEFIT OF S-MMA DETERMINATION

Our findings indicate that the clinical judgment of general practitioners has a higher diagnostic accuracy than s-cobalamin used without clinical information. However, the clinical diagnostic efficiency is still not high. Therefore, we wanted to estimate the diagnostic benefit of using s-MMA determination. The gain in diagnostic accuracy can be expressed as the discrepancy between the clinical diagnosis and the result of the s-MMA determination.

As an example, we can study a case where the s-cobalamin is 100 pmol/L. For this example, we will consider s-MMA >0.376 nmol/L (and s-creatinine within the health-related reference range) as synonymous with cobalamin deficiency. The probability that the patient will receive a supplement is 0.72 (Fig. 3, inset). Among the subjects receiving supplements , the probability of having increased s-MMA is 0.73 (Fig. 3, main panel). Thus, the fraction correctly supplemented (of all patients with an s-cobalamin value of 100 pmol/L) is 0.52 (0.72 × 0.73). The fraction supplemented without having a deficiency is

Table 6. Relationship between the general practitioner's decision to supplement and the clinical diagnosis and cobalamin concentration.

	s-Cobalamin interval, pmol/L				
	0–139	140–169	170–189	190–219	220–299
Percentage supplemented within each cobalamin interval	73	68	30	4	9
Percentage supplemented within each diagnostic category					
Not deficient	0	0	0	0	0
Uncertain diagnosis	29	33	4	0	18
Deficient	100	100	100	100	100

0.20 (0.72 − 0.52). The fraction not supplemented is 0.28 (1 − 0.72; Fig. 3, inset), with a fraction of 0.69 (1 − 0.31; Fig. 3, main panel) having s-MMA within the reference interval. Thus, the fraction of patients correctly not supplemented is 0.19 (0.28 × 0.69), and the fraction wrongly not supplemented, i.e., subjects who should have been supplemented, is 0.09 (0.28 − 0.19). Of all patients with an s-cobalamin value of 100 pmol/L, a fraction of 0.60 had increased s-MMA and a fraction of 0.40 had s-MMA within the reference range. The use of s-MMA as the reference standard therefore implies a fractional increase in true-positive diagnoses of 0.08 (0.60 − 0.52) and a fractional increase in true-negative diagnoses of 0.21 (0.40 − 0.19). This fractional increase in "true" diagnoses is shown in Fig. 4 A.

In our model, we know the fraction of patients with s-MMA >0.376 nmol/L (*A*) for different cobalamin concentrations. If s-MMA is not used as the reference standard, but as a test with a certain sensitivity (*se*) and specificity (*sp*) for diagnosing cobalamin deficiency, samples with s-MMA >0.376 will include samples from patients with true-positive (*TP*) and false-positive (*FP*) diagnoses. Similarly, s-MMA ≤0.376 nmol/L (*B*) will include patients with true-negative (*TN*) and false-negative (*FN*) diagnoses. If we use the following *(40)* formulas:

$$A = TP + FP;\ B = TN + FN;$$

$$se = \frac{TP}{(TP + FN)};\ sp = \frac{TN}{(TN + FP)} \qquad (1)$$

by calculations and rearrangements, we find that:

$$FP = \frac{((B \cdot se \cdot (1 - sp)) - (A \cdot (1 - sp) \cdot (1 - se)))}{(se + sp - 1)};$$

$$TP = A - FP;\ FN = TP \cdot \frac{(1 - se)}{se};\ TN = B - FN \qquad (2)$$

These equations were used to calculate the fractions of true-positive, false-negative, true-negative, and false-positive diagnoses for different sensitivities and specificities for s-MMA. Fig. 4B shows the calculated fractional diagnostic difference in true-negative and true-positive diagnoses between s-MMA and the "clinical diagnosis" (supplementation or not) with the MMA test sensitivity set at 0.95 and the specificity set at 0.97.

The diagnostic performance of metabolite assays (s-MMA and s-tHcy) was reviewed extensively in a recent monograph *(41)*. The sensitivity and specificity of s-MMA are >0.90 in most studies.

cost-benefit estimates of s-MMA measurements

A simple cost-benefit analysis was made to find the s-cobalamin interval where s-MMA should be determined. In this analysis, we compared the costs and benefits of the additional use of s-MMA in diagnosing cobalamin deficiency:

$$Benefit\ of\ conventional\ diagnostic\ procedures = w \cdot (TP - FN) + (TN - FP) \qquad (3)$$

$$Benefit\ of\ using\ s\text{-}MMA > 0.376 = w \cdot (TP - FN) + (TN - FP) - costs\ of\ s\text{-}MMA\ assay \qquad (4)$$

We defined in absolute values the benefit of giving supplements or not giving supplements to a deficient patient as equal (*a*). In comparison, the cost of treating and the benefit of not treating nondeficient patients are also defined as equal (*b*). The values for *w* in Eqs. 3 and 4 are the different ratios of *a* to *b*. The relative benefit between the conventional diagnostic procedures and the use of s-MMA for the diagnosis of cobalamin deficiency is obtained by plotting the ratio of Eq. 3 to Eq. 4. When this ratio is <1, s-MMA should be requested. When the ratio is >1, s-MMA should not be requested. The ratio when the costs of the analysis are set to 5% of *b* and the sensitivity and specificity for s-MMA as the test are 100% is shown in

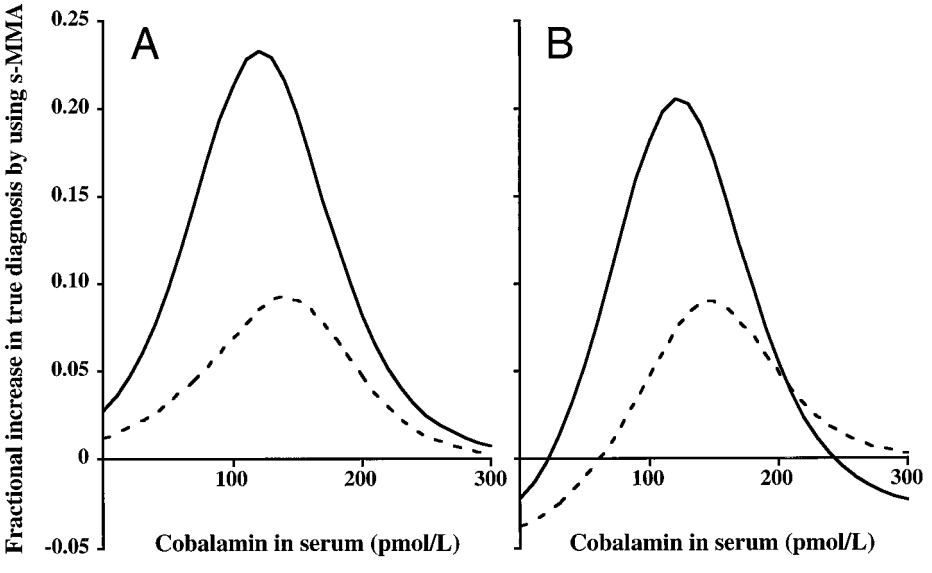

Fig. 4. Changes in the fraction of true-positive and true-negative diagnoses when the decision by general practitioners to give patients supplements is replaced by s-MMA as the reference standard.

(*A*), the fractional increase when s-MMA is used as reference standard; (*B*), the corresponding results when s-MMA has a sensitivity of 0.95 and a specificity of 0.97 (see text). (————), the difference between the true-negative rate when s-MMA is used as reference standard and the true-negative rate when the general practitioners' clinical diagnostic conclusions (supplementation or not) are used as the reference; (– – – –), the corresponding difference in the true-positive diagnoses.

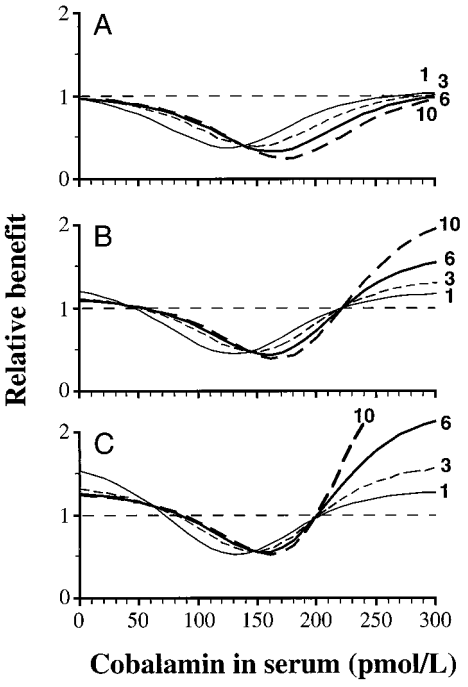

Fig. 5. Relative benefits of using the conventional diagnostic procedures and using s-MMA for the diagnosis of cobalamin deficiency.

Benefit ratio <1 indicates that s-MMA should be requested. (*A*), s-MMA is considered the reference standard with a sensitivity of 100% and a specificity of 100%. (*B*), s-MMA is a test with a sensitivity of 0.95 and a specificity of 0.97. (*C*), s-MMA is a test with a sensitivity of 0.90 and a specificity of 0.95. The calculations as well as the weights used are explained in the text.

Fig. 5A. We can see that the test should be requested in most cases up to s-cobalamin values of 250 pmol/L. By using the Eqs. 1 and 2, we can simulate the changes of relative benefit of the MMA-test giving the assay different sensitivities and specificities. With a sensitivity of 95% and a specificity of 97% for s-MMA, which is presumably a realistic assumption *(35)*, s-MMA should be requested if a patient has s-cobalamin values between 60 and 220 pmol/L. The conclusion is more or less independent of w (Fig. 5B). With a sensitivity of 90% and a specificity of 95%, s-MMA should be requested for s-cobalamin values between 90 and 200 pmol/L (Fig. 5C). Even if the costs of the s-MMA-assay are changed to 20% of the false positives (b), the effects on the conclusions of our model are minor (data not shown), and s-MMA should be requested at s-cobalamin values between 90 and 190 pmol/L. Thus, the model is rather robust for small changes in the diagnostic accuracy of s-MMA, as well as for alterations in the cost-benefit weights and different estimates of the costs of the MMA analysis.

Conclusions

In general practice, s-cobalamin is usually requested on the basis of nonspecific indications or vague symptoms. When general practitioners decide to supplement patients, they primarily take into consideration the cobalamin value and whether the patient is anemic. General practitioners often postpone making a definitive diagnosis when s-cobalamin is in the lower reference range.

Our data emphasize the poor diagnostic utility of low and low-normal s-cobalamin assay results and call for more sensitive and specific markers of cobalamin deficiency. s-MMA meets these criteria, and if used, our data suggest routine measurement in patients when s-cobalamin is >60–90 pmol/L and <200–220 pmol/L, depending on the diagnostic accuracy of s-MMA.

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