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The marker of cobalamin deficiency, plasma methylmalonic acid, correlates to plasma creatinine

A. M. HVAS[1], S. JUUL[2], L. U. GERDES[3] & E. NEXØ[3]
From the [1]Department of Haematology, AAS, Aarhus University Hospital; the [2]Department of Epidemiology and Social Medicine, Aarhus University; and the [3]Department of Clinical Biochemistry, AAS/AKH, Aarhus University Hospital, Aarhus, Denmark

Abstract. Hvas AM, Juul S, Gerdes LU, Nexø E (Aarhus University and Aarhus University Hospital, Aarhus, Denmark). The marker of cobalamin deficiency, plasma methylmalonic acid, correlates to plasma creatinine. *J Intern Med* 2000; **247:** 507–512.

Objective. To examine the relationship between the two diagnostic tests, plasma methylmalonic acid and plasma cobalamins, and their association with plasma creatinine, age and sex.
Design. Cross-sectional study of simultaneous laboratory measurements.
Setting. County of Aarhus, Denmark.
Subjects. Records on 1689 patients who had their first plasma methylmalonic acid measurement during 1995 and 1996, and who had a simultaneous measurement of plasma cobalamins. Plasma creatinine values measured within a week of measurements of plasma methylmalonic acid and plasma cobalamins were available for 1255 of the patients.
Main outcome measures. Predictors of variation in plasma methylmalonic acid; plasma cobalamins, plasma creatinine, age and sex.
Results. Plasma methylmalonic acid was positively correlated with plasma creatinine, even for plasma creatinine within the normal range. These associations remained in a multiple regression analysis. For plasma cobalamins below 200 pmol L^{-1}, there was a strong negative correlation between plasma methylmalonic acid and plasma cobalamins, whilst the association was weak for higher plasma cobalamin levels. Plasma methylmalonic acid increased and plasma cobalamins decreased with age.
Conclusions. The strong correlation between plasma methylmalonic acid and plasma creatinine suggests that plasma creatinine – also within the normal range – must be taken into consideration when interpreting plasma methylmalonic acid.

Keywords: creatinine, diagnostic test, methylmalonic acid, vitamin B_{12} deficiency, vitamin B_{12}.

Introduction

The usefulness of plasma cobalamin in diagnosing cobalamin deficiency has been extensively discussed and it is generally accepted that it cannot be used as a sole criterion for cobalamin deficiency [1–6]. Plasma methylmalonic acid (P-MMA) has been proposed to be a more specific and sensitive marker than plasma cobalamin [4, 7–9] and has therefore been increasingly used as an alternative or supplementary test during the past 10 years.

It has been suggested that cobalamin deficiency should be defined as an increased P-MMA that decreases upon treatment with cobalamin [6, 10, 11]. This criterion is not generally accepted, however, and some have expressed the concern that this use of P-MMA may result in treatment of biochemical changes of no clinical importance [12, 13].

P-MMA is elevated in patients with impaired kidney function, as expressed by elevated plasma creatinine [10, 14–16]. However, little is known about the association between P-MMA and plasma creatinine in the whole range of values of plasma creatinine. This is an important issue since one precondition for P-MMA to be a superior test for diagnosis of cobalamin deficiency is that it is unaffected, or affected in a simple way, by factors other than cobalamin deficiency.

We examined the relationship between P-MMA

and plasma cobalamins, age, sex and plasma creatinine. Our most important finding is a strong correlation between P-MMA and plasma creatinine over the entire range of values for plasma creatinine.

Materials and methods

All records with P-MMA, plasma cobalamins and plasma creatinine were extracted from the laboratory information systems at Aarhus University Hospital. We selected the records for 1689 patients who had P-MMA and plasma cobalamins requested on the same day during 1995–96. Only the first P-MMA measurement for each patient was included. The purpose of this restriction was to obtain data representing unselected and untreated patients with some suspicion of cobalamin deficiency. Plasma creatinine values measured within 7 days before or after the index measurements were available for 1255 patients.

P-MMA was determined by gas chromatography-mass spectrometry [17], and the reference interval for P-MMA was 0.08–0.28 μmol L^{-1} [18]. The coefficient of variation was 7.9% for P-MMA [17]. Plasma cobalamins were determined using human intrinsic factor as binding protein [19], and the reference interval was 200–600 pmol L^{-1} [20]. Plasma creatinine was measured with Jaffés method using a Roche Cobas Integra 700 autoanalyser, or by a dry-slide enzymatic method using a Vitros 950 autoanalyser (Johnson & Johnson Clinical Diagnostics). The reference interval was 55–120 μmol L^{-1}. The coefficient of variation for plasma creatinine was about 2%.

We used Mantel–Haenszel's test for trend to examine the association between ordinal variables, and Pearson's correlation and multiple linear regression analysis to examine the association between continuous variables. Log transformations were used when appropriate. Data were analysed with SPSS.

Results

Table 1 shows the distributions of age, P-MMA, plasma cobalamins and plasma creatinine. The majority of patients were above 60 years old, and 62% were females. Thirty per cent had a concentration of P-MMA above 0.28 μmol L^{-1} and 18% had plasma cobalamins below 200 pmol L^{-1}. Ninety per cent of the patients had plasma creatinine below 120 μmol L^{-1}.

Fifty-nine per cent of the P-MMA tests were requested by physicians in hospitals and 41% by general practitioners. There were no substantial differences in test results and age distribution between the hospital and general practitioner groups.

Figure 1 and Table 2 show the joint distribution of P-MMA and plasma cobalamins. The correlation between P-MMA and plasma cobalamins was strong for plasma cobalamins below 200 pmol L^{-1} ($r = -0.59$ for log-transformed values, $P < 0.001$), but weak for higher plasma cobalamin values ($r = -0.05$, $P = 0.09$). This finding did not vary substantially with age and gender (data not shown).

Nine per cent of the patients had abnormal values for both tests (P-MMA $>$ 0.28 μmol L^{-1} and plasma cobalamins $<$ 200 pmol L^{-1}), and 62% had normal values for both. Thus, discrepancies between P-MMA and plasma cobalamin values were quite frequent. Forty-six per cent of the patients with low plasma cobalamins ($<$ 200 pmol L^{-1}) had normal P-MMA values ($<$ 0.29 μmol L^{-1}); 25% of the patients with normal plasma cobalamins had elevated P-MMA values.

Plasma cobalamins declined and P-MMA and plasma creatinine increased with age. There was a

Table 1 Distribution of age and test values in the study group ($n = 1689$)

	Percentile						
	Minimum	5	25	50	75	95	Maximum
Age (years)	9	28	49	66	77	87	100
P-MMA (μmol L^{-1})	0.04	0.09	0.16	0.22	0.31	0.69	60.5
Plasma cobalamins (pmol L^{-1})	27	135	223	292	391	690	700
Plasma creatinine (μmol L^{-1}) ($n = 1255$)	41	59	71	83	98	145	825

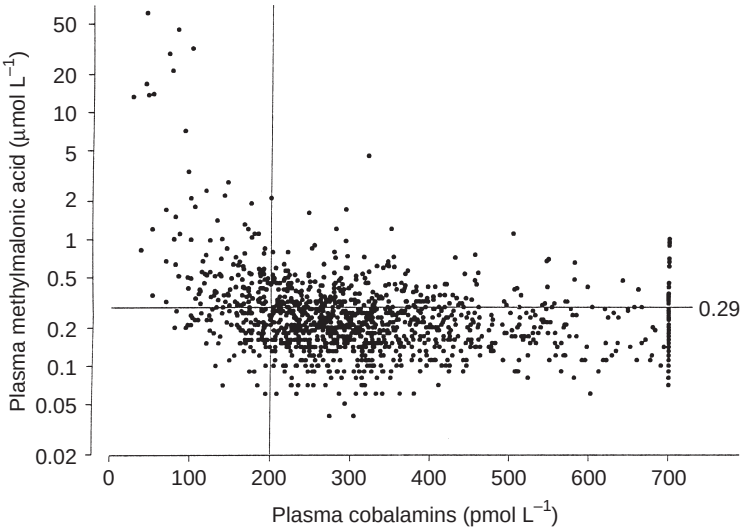

Fig. 1 Distribution of plasma methylmalonic acid and plasma cobalamins amongst 1689 patients.

weak positive association of plasma cobalamins with plasma creatinine ($r = 0.05$, $P = 0.06$). The dependency of P-MMA on plasma cobalamins, plasma creatinine, age and sex was further analysed with multiple linear regression, Table 3. Plasma cobalamins, plasma creatinine and age were strong and independent predictors of P-MMA, whilst sex was a weak predictor. Thirty-one per cent of the variation of P-MMA could be explained by these variables. Seventeen outliers (P-MMA > 2.00 μmol L^{-1}) were excluded, and after this exclusion, residual analysis demonstrated a good fit to the regression model. No statistical interactions (effect modification) were found, as the coefficients estimated were similar for old and young, males and females, patients with high and low plasma creatinine, and high and low plasma cobalamins.

The regression coefficients were used to predict the level of P-MMA from plasma cobalamins, plasma creatinine, sex and age. To illustrate the effect of plasma cobalamins and plasma creatinine, Fig. 2 shows the predicted P-MMA (geometric mean) at various levels of plasma creatinine and plasma cobalamins for 70-year-old women. A 10% decline in plasma cobalamins corresponded to a 3% increase in P-MMA, whilst a 10% increase in plasma creatinine corresponded to a 6% increase in P-MMA.

For a patient with low plasma cobalamins (150 pmol L^{-1}) and a low plasma creatinine (50 μmol L^{-1}), the predicted P-MMA was within the reference interval (0.22 μmol L^{-1}), whilst for a patient with normal plasma cobalamins (250 pmol L^{-1}) and a high normal plasma creatinine (100 μmol L^{-1}), the predicted P-MMA was just above the reference interval (0.29 μmol L^{-1}).

The association between plasma creatinine and P-MMA is also illustrated in Table 4. In patients with low and with normal plasma cobalamins, the proportion with elevated P-MMA increased with the level of plasma creatinine. Thus the discrepan-

Table 2 Plasma methylmalonic acid (P-MMA) as a function of plasma cobalamins. The number and percentage of patients are shown for each interval of plasma cobalamins

Plasma cobalamins (pmol L^{-1})	P-MMA (μmol L^{-1})				
	– 0.28	0.29–0.44	0.45–0.99	1.00+	Total
< 149	32 (28)	26 (23)	28 (25)	27 (24)	113 (100)
150–199	107 (58)	40 (22)	32 (17)	7 (4)	186 (100)
200+	1040 (75)	242 (17)	97 (7)	11 (1)	1390 (100)
Total	1179 (70)	308 (18)	157 (9)	45 (3)	1689 (100)

χ^2trend = 222; d.f. = 1; $P < 0.001$.

Table 3 Dependency of plasma methylmalonic acid (P-MMA) on plasma creatinine, plasma cobalamins, age and sex. Linear regression analysis. Dependent variable: ln (P-MMA [μmol L^{-1}])

Independent variable	Unadjusted coefficients (95% CI)	Mutually adjusted coefficients (95% CI)
Constant		– 2.909 (– 3.398, – 2.421)
ln (plasma cobalamins [pmol L^{-1}])	– 0.313 (– 0.382, – 0.245)	– 0.324 (– 0.384, – 0.265)
ln (plasma creatinine [μmol L^{-1}])	0.653 (0.561, 0.746)	0.603 (0.513, 0.693)
Age (per year)	0.013 (0.011, 0.014)	0.010 (0.008, 0.011)
Sex (male)	0.026 (– 0.040, 0.091)	– 0.065 (– 0.123, – 0.008)

1238 patients with plasma creatinine within 1 week of P-MMA, and P-MMA < 2.00 μmol L^{-1}; $R^2 = 0.31$.

cies shown in Table 2 between P-MMA and plasma cobalamin findings can partly, but not entirely, be explained by variations in plasma creatinine. Keeping in mind the absence of a definite criterion of cobalamin deficiency, the results in Table 4 express the sensitivity and specificity of P-MMA > 0.28 μmol L^{-1} in predicting plasma cobalamins < 200 pmol L^{-1}. With plasma creatinine below 80 μmol L^{-1}, the sensitivity was 44% and the specificity 83%, whilst with plasma creatinine in the interval 100–119 μmol L^{-1}, the sensitivity was 73% and the specificity 74%.

Discussion

A large number of simultaneously determined plasma cobalamins and P-MMA values allowed a thorough analysis of the relationship between the two variables and of the influence of possible confounding factors such as sex, age and plasma creatinine.

Our most prominent finding is a strong association between P-MMA and plasma creatinine also within the normal range for plasma creatinine. In contrast, no significant association was found between plasma cobalamins and plasma creatinine. We believe the results to be of special relevance for patients with decreased plasma cobalamins. In these patients, P-MMA may be within the current reference interval if plasma creatinine is low-normal (illustrated in Fig. 2). Our results suggest that plasma creatinine needs to be taken into consideration when interpreting P-MMA.

Some have suggested that normal levels of both P-MMA and plasma homocysteine rule out clinically significant cobalamin deficiency [16, 21]. In the light of our study, we agree with others who state that we do not yet know how confidently we can

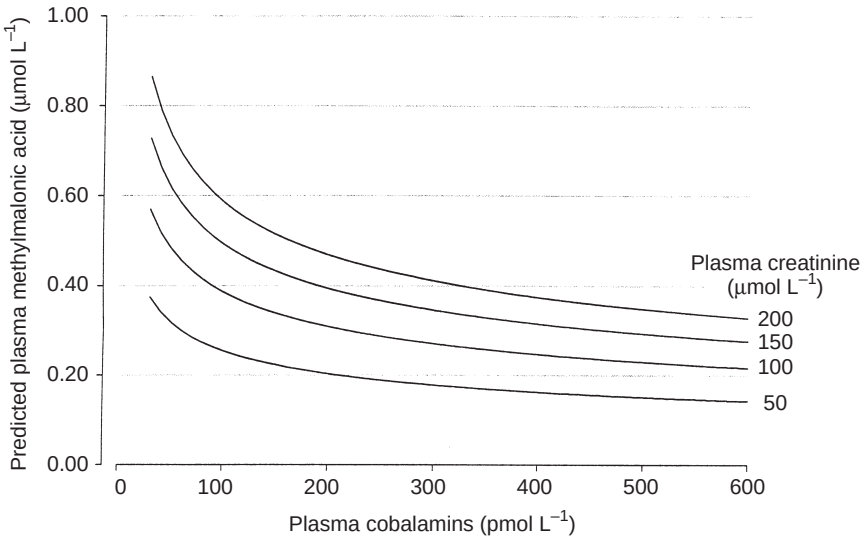

Fig. 2 Predicted plasma methylmalonic acid from plasma creatinine and plasma cobalamins amongst 70-year-old female patients.

Table 4 Proportion of patients with plasma methylmalonic acid > 0.28 μmol L^{-1}, by plasma cobalamins and plasma creatinine

Plasma creatinine (μmol L^{-1})	Plasma cobalamins		
	< 200 pmol L^{-1}	≥ 200 pmol L^{-1}	Total
< 79	42/95 (44)	74/433 (17)	116/528 (22)
80–99	48/78 (62)	84/359 (23)	132/437 (30)
100–119	19/26 (73)	36/137 (26)	55/163 (34)
120+	17/19 (89)	78/108 (72)	95/127 (75)
Total	126/218 (58)	272/1037 (26)	398/1255 (32)

exclude cobalamin deficiency disease when plasma cobalamins are low and P-MMA is normal [13].

In agreement with some previous studies [20, 22–24], we observed decreasing plasma cobalamins and increasing levels of P-MMA with advancing age. The influence of sex was marginal. As shown by previous studies on smaller samples [9, 21, 22] we found a significant correlation between plasma cobalamins and P-MMA at plasma cobalamin levels below 200 pmol L^{-1}. However, the discrepancy was substantial between results obtained by the two tests. The relationship between P-MMA and plasma creatinine may explain some of this discrepancy, but is unlikely to be the only explanation.

Elevated P-MMA with normal plasma cobalamins might reflect poor specificity of P-MMA or poor sensitivity of plasma cobalamins in diagnosing cobalamin deficiency. It has been argued for years that the sensitivity of plasma cobalamins is low [1, 2] and it is well known that P-MMA may be high in conditions not related to cobalamin deficiency. An increased P-MMA is seen in renal insufficiency [14–16], thyroid disease, small bowel bacterial overgrowth, and conditions with haemoconcentration [25], but the frequency of non-specific elevations in P-MMA due to these reasons is unknown. As noted by Chanarin and Metz [12] the specificity of P-MMA has not been adequately evaluated.

A recent study by Lindgren *et al.* [26] did not find a higher specificity and sensitivity of increased P-MMA compared with low plasma cobalamins for identifying patients with conditions compatible with cobalamin malabsorption. These findings are in strong contrast with earlier studies claiming much higher sensitivity of abnormal P-MMA concentrations compared with low plasma cobalamins [22, 27, 28]. The study by Lindgren *et al.* [26] and our findings emphasize that the interpretation of P-MMA is still a key question.

In conclusion, the interpretation of P-MMA and plasma cobalamins is still uncertain, as is the strategy for diagnosing cobalamin deficiency. Obviously our study does not enable one to determine whether the combination of P-MMA and plasma creatinine could give clinically more relevant information than do plasma cobalamin measurements. But the study strongly suggests that plasma creatinine must be included when assessing P-MMA, and reference intervals not taking age and plasma creatinine into account can be misleading.

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References

1 England JM, Linnell JC. Problems with the serum vitamin B12 assay. *Lancet* 1980; **ii**: 1072–73.
2 Herbert V, Colman N, Palat D *et al.* Is there a 'gold standard' for human serum vitamin B12 assay? *J Lab Clin Med* 1984; **104**: 829–41.
3 Carmel R. Pernicious anemia. The expected findings of very low serum cobalamin levels, anemia, and macrocytosis are often lacking. *Arch Intern Med* 1988; **148**: 1712–14.
4 Allen RH, Stabler SP, Savage DG, Lindenbaum J. Diagnosis of cobalamin deficiency I. Usefulness of serum methylmalonic acid and total homocysteine concentrations. *Am J Hematol* 1990; **34**: 90–98.
5 Green R. Screening for vitamin B12 deficiency: caveat emptor. *Ann Intern Med* 1996; **124**: 509–11.
6 Nexø E, Hansen M, Rasmussen K, Lindgren A, Grasbeck R. How to diagnose cobalamin deficiency. *Scand J Clin Lab Invest Suppl.* 1994; **219**: 61–76.
7 Stabler SP, Marcell PD, Podell ER, Allen RH, Lindenbaum J. Assay of methylmalonic acid in the serum of patients with cobalamin deficiency using capillary gas chromatography-mass spectrometry. *J Clin Invest* 1986; **77**: 1606–12.
8 Lindenbaum J, Savage DG, Stabler SP, Allen RH. Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. *Am J Hematol* 1990; **34**: 99–107.
9 Moelby L, Rasmussen K, Jensen MK, Pedersen KO. The relationship between clinically confirmed cobalamin deficiency and serum methylmalonic acid. *J Intern Med* 1990;

228: 373–78.
10 Lindenbaum J, Healton EB, Savage DG *et al.* Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med* 1988; **318**: 1720–28.
11 Moelby L, Nielsen G, Rasmussen K, Jensen MK, Pedersen KO. Metabolic cobalamin deficiency in patients with low to low-normal plasma cobalamins. *Scand J Clin Lab Invest* 1997; **57**: 209–16.
12 Chanarin I, Metz J. Annotation. Diagnosis of cobalamin deficiency: the old and the new. *Br J Haematol* 1997; **97**: 695–700.
13 Schilling RF. Who has vitamin B_{12} deficiency? *Proc Assoc Am Phys* 1996; **108**: 68–70.
14 Rasmussen K, Vyberg B, Pedersen KO, Brochner-Mortensen J. Methylmalonic acid in renal insufficiency: evidence of accumulation and implications for diagnosis of cobalamin deficiency. *Clin Chem* 1990; **36**: 1523–24.
15 Moelby L, Rasmussen K, Rasmussen HH. Serum methylmalonic acid in uraemia. *Scand J Clin Lab Invest* 1992; **52**: 351–54.
16 Stabler SP. Screening the older population for cobalamin (vitamin B_{12}) deficiency. *J Am Geriatr Soc* 1995; **43**: 1290–97.
17 Rasmussen K. Solid-phase sample extraction for rapid determination of methylmalonic acid in serum and urine by a stable-isotope-dilution method. *Clin Chem* 1989; **35**: 260–64.
18 Rasmussen K, Moller J, Lyngbak M, Pedersen AM, Dybkjaer L. Age- and gender-specific reference intervals for total homocysteine and methylmalonic acid in plasma before and after vitamin supplementation. *Clin Chem* 1996; **42**: 630–36.
19 Nexø E, Gimsing P. Insolubilized pure human intrinsic factor used for quantitation of cobalamins in serum. *Scand J Clin Lab Invest* 1981; **41**: 465–68.
20 Nexø E. Variation with age of reference values for P-cobalamins. *Scand J Haematol* 1983; **30**: 430–32.
21 Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. *Am J Med* 1994; **96**: 239–46.
22 Lindenbaum J, Rosenberg IH, Wilson PW, Stabler SP, Allen RH. Prevalence of cobalamin deficiency in the Framingham elderly population. *Am J Clin Nutr* 1994; **60**: 2–11.
23 Elsborg L, Lung V, Bastrup-Madsen P. Serum vitamin B12 levels in the aged. *Acta Med Scand* 1976; **200**: 309–14.
24 Nilsson-Ehle H, Landahl S, Lindstedt G *et al.* Low serum cobalamin levels in a population study of 70- and 75-year-old subjects. Gastrointestinal causes and hematological effects. *Dig Dis Sci* 1989; **34**: 716–23.
25 Norman EJ, Martelo OJ, Denton MD. Cobalamin (vitamin B12) deficiency detection by urinary methylmalonic acid quantitation. *Blood* 1982; **59**: 1128–31.
26 Lindgren A, Swolin B, Nilsson O, Johansson KW, Kilander AF. Serum methylmalonic acid and total homocysteine in patients with suspected cobalamin deficiency: a clinical study based on gastrointestinal histopathological findings. *Am J Hematol* 1997; **56**: 230–38.
27 Pennypacker LC, Allen RH, Kelly JP *et al.* High prevalence of cobalamin deficiency in elderly outpatients. *J Am Geriatr Soc* 1992; **40**: 1197–204.
28 Joosten E, van den Berg A, Riezler R *et al.* Metabolic evidence that deficiencies of vitamin B-12 (cobalamin), folate, and vitamin B-6 occur commonly in elderly people. *Am J Clin Nutr* 1993; **58**: 468–76.

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Correspondence: Anne-Mette Hvas, Department of Haematology, Aarhus University Hospital, Aarhus Amtssygehus, Tage Hansens Gade 2, Dk-8000 Aarhus C, Denmark (fax: + 45 89497598; e-mail: Anne_Mette.Hvas@aas.auh.dk).