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Clinical significance of low cobalamin levels in older hospital patients

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Abstract

Background: It is still a commonly held belief that many of the frequently found low cobalamin (Cbl, vitamin B12) levels in older people do not represent deficiency and are therefore without clinical significance and should not be treated. In this study this notion will be challenged.

Methods: In this prospective observational cohort design we studied 28 patients aged 65 years and older with low plasma Cbl (≤ 150 pmol/l). A number of haematological (Hb, MCV, five- and six-lobed granulocytes), metabolic (plasma levels of methylmalonic acid and homocysteine), and gastrointestinal (plasma pepsinogen A and C and protein-bound and free Cbl absorption) parameters, and the response to Cbl treatment, were measured. Cbl deficiency was considered to be present when at least one of the following three criteria was fulfilled: (1) haematological or metabolic abnormalities compatible with Cbl deficiency; (2) Cbl malabsorption or atrophic gastritis; (3) a response to Cbl supplementation.

Results: Haematological or metabolic abnormalities were identified in 27 patients. Atrophic gastritis and Cbl malabsorption were identified in, respectively, 15 and 23 patients. Each treated patient showed a haematological or metabolic response to Cbl supplementation. All patients were considered Cbl deficient: 18 patients (64%) fulfilled three criteria of Cbl deficiency, eight (29%) fulfilled two criteria and two (7%) fulfilled one criterion.

Conclusions: According to the generally accepted and a wide variety of criteria, we found that older patients with low Cbl levels were cobalamin deficient. Therefore, these patients should receive Cbl supplementation.

Keywords: Methylmalonic acid; Homocysteine; Absorption; Gastritis; Supplementation

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Introduction

Measurement of plasma cobalamin (Cbl) levels is the most frequently used method for the detection of cobalamin deficiency. However, ever since the introduction of the Cbl assay, questions have been raised about the clinical significance of low plasma cobalamin levels in patients without anaemia, macrocytosis, or other symptoms compatible with Cbl deficiency [1]. It has been argued that the cobalamin assay has a low specificity and that low plasma Cbl levels in apparently asymptomatic patients are without any clinical significance [2–5].

In other studies, the existence of mild (or subtle or atypical) cobalamin deficiency states has been postulated (reviewed in Ref. [6]). Mild Cbl deficiency is defined as metabolic evidence of deficiency without the overt manifestations of anaemia or neurologic disease. Metabolic evidence refers to increased levels of methylmalonic acid (MMA) or total homocysteine (tHcy). Plasma levels of MMA and tHcy have proven to be highly sensitive indicators of tissue cobalamin deficiency [7]. Protein-bound or food Cbl malabsorption, not detected with the standard Schilling test, is a frequent cause of mild Cbl deficiency [8]. Whether an early stage, a transient phenomenon, or a prolonged condition in its own right, mild cobalamin deficiency is a finding with a high prevalency in the elderly.

In view of the many and on-going questions on screening, diagnosis and treatment [9,10], we investigated the significance of low Cbl levels in older patients by extensive examination of all haematological, metabolic and gastrointestinal parameters relevant for Cbl deficiency. Currently available diagnostic techniques such as measurement of plasma concentrations of MMA, tHcy, pepsinogen A and C and protein-bound and free Cbl absorption were applied. In addition, we examined the response of the haematological and metabolic parameters to Cbl supplementation. Cbl deficiency was considered to be present when at least one of the following criteria was fulfilled: (1) the presence of haematological or metabolic abnormalities (anaemia, macrocytosis, neutrophilic hypersegmentation, elevated plasma MMA or tHcy levels) not attributable to other clinical conditions; (2) the presence of Cbl malabsorption or associated gastric abnormality, i.e. atrophic gastritis; (3) the presence of a haematological or metabolic response to Cbl supplementation.

Materials and methods

Sample

During a recruitment period of 1.5 years, all patients older than 65 years from the Departments of Geriatric Medicine, Neurology and Internal Medicine with low plasma Cbl levels (≤ 150 pmol/l) were considered for participation in the study by weekly reviews of the laboratory log book. The following exclusion criteria were applied: inability to comply with the protocol for urine collection (altered mental status, urinary incontinence, chronic renal failure), critical illness, normal plasma Cbl level on the second Cbl assay, or treatment with Cbl before the start of the study. Plasma Cbl level was low in 128 of 887 patients (14.4%). Fifty-two patients were excluded. Of the remaining 76 eligible patients, 48 declined participation mainly because they considered the protocol was to cumbersome. Therefore, 28 patients participated.

Twenty-five healthy older volunteers (median age 74, range 66–87 years, plasma Cbl >150 pmol/l), recruited from the local community by means of advertisements in local newspapers, served as controls for the determination of reference values of plasma MMA, tHcy and the protein-bound cobalamin and free cobalamin absorption tests [11]. The following exclusion criteria were applied: inability to comply with the protocol for urine collection (altered mental status, urinary incontinence, chronic renal failure), vegetarianism, liver disease, alcoholism, previous stomach or intestinal surgery, intestinal diseases, chronic diarrhea, unexplained weight loss, anaemia, cobalamin or folate deficiency in the past, ongoing treatment with cobalamin, folic acid, antacids, antibiotics, chloral hydrate, vitamin C, anticonvulsants, metformin, potassium salt or colestyramine.

Protocol

The Committee for Experimental Research with Humans of the University Hospital Nijmegen ap-

proved the protocol. Written informed consent was obtained from all participants. Medical history, including use of medication, was obtained. The Geriatric Depression Scale (GDS [12]), the Mini Mental State Examination (MMSE [13]), and the Barthel ADL Index [14] were used to assess the cognitive and functional abilities. Blood samples were collected after overnight fast. Protein-bound cobalamin absorption (PCA) and free cobalamin absorption (FCA, the first stage of the Schilling test) were assessed as described earlier [11]. Protein-bound Cbl absorption was assessed by the 48-h urinary excretion method following oral administration of scrambled egg yolk labeled in vivo with ^{57}Co-cyanocobalamin by injecting an egg-laying hen with ^{57}Co-cyanocobalamin. PCA less than 9.4% and FCA less than 19.7% (5th percentiles in controls) were considered abnormal. Abnormal FCA tests were classified as follows [7]: proven pernicious anaemia (presence of serum antibodies against intrinsic factor (IF)); probable pernicious anaemia (abnormal FCA test with other features compatible with pernicious anaemia such as elevated serum gastrin levels and severe atrophic gastritis); intestinal malabsorption (normal gastric status and/or no correction of FCA by IF).

After completion of the absorption studies, 22 patients received hydroxocobalamin injections from their attending physicians, at least 1000 μg per month. The haematological and metabolic parameters of 18 patients could be reassessed after Cbl supplementation (two patients refused and two patients had died, median follow-up 5.5 months, range 3 to 11 months).

Laboratory techniques

Plasma Cbl and folate in erythrocytes were measured by competitive radioisotope binding techniques (Solid Phase Boil Dualcount, Diagnostic Products Corporation, Los Angeles, CA, USA). The reference values for Cbl in our laboratory are 150–750 pmol/l. Blood cell counts were analysed on an H1 (Technicon). Blood smears were blinded for review by an experienced technician who was unaware of any clinical or laboratory finding. The number of five- and six-lobed granulocytes per 100 polymorphonuclear neutrophils was scored. Hypersegmentation of neutrophils was defined as more than five five-lobed or one six-lobed granulocyte per 100 polymorphonuclear neutrophils [7]. Serum IF antibodies were measured by radioimmunoassay. Plasma MMA concentration was determined by stable isotope dilution capillary gas chromatography–mass spectrometry [15]. In the preparation procedure of the samples, solvent extraction with ethylacetate was used instead of solid-phase extraction. Plasma tHcy concentration was measured by the automated HPLC method with reverse-phase separation and fluorescent detection [16]. Plasma MMA and tHcy levels >0.32 and >19.9 μmol/l (5th percentile in controls), respectively, were considered as elevated. Plasma gastrin, pepsinogen A and C concentrations were measured by radioimmunoassay [17–19]. Mild to moderate atrophic gastritis is defined as a ratio of pepsinogen A to C of <1.6 combined with a pepsinogen A level of >17 mg/l. Severe atrophic gastritis was defined as a ratio of pepsinogen A to C of <1.6 combined with a pepsinogen A level of <17 mg/l [19].

Statistical analysis

Results are presented as medians with 25th and 75th percentile. The 5th or 95th percentiles, determined by the non-parametric method with 90% reliability, in controls were used for the determination of the reference values. The Mann–Whitney Test was used for comparing variables of unpaired samples. The Wilcoxon's Signed-Rank Test was used for comparing variables of paired samples. $P<0.05$ was considered as statistically significant.

Results

Patients

The clinical characteristics of the patients are presented in Table 1. Twenty-one patients were outpatients and 20 patients were living independently. Seventeen patients were independent for ADL. Eleven patients had one chronic condition and 21 used three or less medications per day. Four patients had a MMSE score below the cut-off point of 24, indicating cognitive dysfunction, and four patients

Table 1
Clinical characteristics of older patients with reduced plasma cobalamin levels[a]

	Patients ($n = 28$)
Age, median (range)	74 (66–90)
Male/female	10/18
Number of chronic conditions, median (range)	1 (0–3)
Number of medications, median (range)	3 (0–7)
Mini Mental State Examination score [13], median (range)	30 (14–30)
Geriatric Depression Scale score [12], median (range)	7 (1–26)
Barthel ADL-Index score [14], median (range)	20 (6–20)

[a] Chronic conditions: diabetes, hypertension, stroke, malignancy, vascular disease, arthritis/arthrosis, lung disease, osteoporosis, inflammatory bowel disease.

had a GDS score above the cut-off point of 14, indicating depression. Two patients judged their health as very bad, two as bad, 10 as fair, 11 as good and three as very good. For each patient, the relevant medical history, presenting problems, main laboratory findings, results of the Cbl absorption tests and the response to Cbl supplementation are presented in Table 2. The Cbl assay was requested for the following reasons: screening ($n = 19$); neurological abnormalities ($n = 4$); haematological abnormalities ($n = 4$); previously low Cbl level ($n = 1$). Plasma Cbl was <75 pmol/l in seven, between 75 and 100 pmol/l in 10 and between 100 and 150 pmol/l in 11 patients.

Haematological and metabolic abnormalities

Haematological or metabolic abnormalities were identified in 27 patients. None of the patients had macrocytic anaemia. Only three patients had anaemia, which was explained by iron deficiency in case 3. Macrocytosis (MCV >100 fl) was present in five patients (cases 6, 8, 10, 13 and 21). In case 13, this was, at least partially, attributable to azathioprine therapy. Absence of macrocytosis was explained by iron deficiency in cases 1 and 3. More than 5% five-lobed neutrophils were found in 20 (78%) and six-lobed neutrophils were found in 15 (53.6%) patients.

Plasma MMA was elevated in 23 (82%) and tHcy levels were elevated in 18 (64%) patients. Both metabolite levels were elevated in 17 patients (61%). Two patients (cases 15 and 21) with elevated MMA and tHcy levels had high serum creatinine levels (147 and 139 μmol/l). Nevertheless, the metabolites normalised after Cbl supplementation in case 21 (case 15 not available). Three patients (cases 6, 12 and 7) had low E-folate levels. However, these patients had elevated plasma tHcy levels normalising after Cbl supplementation. Only four patients (cases 11, 13, 22 and 26) had normal metabolite levels. Three had Cbl malabsorption (cases 11, 13 and 26) and two (cases 11 and 13) had haematological abnormalities which responded to Cbl supplementation. Case 22, without Cbl malabsorption or haematological findings, had severe atrophic gastritis as indicated by the plasma pepsinogens and gastrin levels. Plasma metabolite levels did not differ significantly in patients for whom Cbl was requested for screening or for specific reasons.

Gastrointestinal abnormalities

Cbl malabsorption was identified in 23 patients (82%). Fifteen of 27 patients had an abnormal FCA test indicating pernicious anaemia ($n = 9$; proven in cases 2, 6 and 8 and probable in cases 5, 9, 14, 17, 24 and 28), intestinal malabsorption ($n = 3$; cases 7, 11 and 13), and unknown in three patients (cases 1, 3 and 25). All but two of the patients with abnormal FCA tests had an abnormal PCA test (cases 1 and 25).

Of the 12 patients with normal FCA, eight had abnormal PCA, indicating food Cbl malabsorption (cases 12, 15, 16, 19, 20, 21, 23 and 26). Abnormal PCA tests could be explained by atrophic gastritis in three patients (cases 19, 23 and 26). Five patients absorbed Cbl normally. Four of them had atrophic gastritis (cases 4, 18, 22 and 27) and four (cases 4, 10, 18 and 27) had elevated metabolites.

Atrophic gastritis, indicated by a low pepsinogen A to C ratio, was found in 15 (54%) patients, of whom 11 (39%) had severe atrophic gastritis. Plasma gastrin was elevated in 10 of the patients with atrophic gastritis. Twelve of the patients with atrophic gastritis had Cbl malabsorption.

Table 2
Medical history, presenting problems, main laboratory findings, results of the cobalamin absorption tests and response to cobalamin therapy[a]

Case No. age/sex	Relevant history	Presenting problems	Cbl (pmol/l)	Hb (mmol/l)	MCV (fl)	Five-lobed neutro's	Six-lobed neutro's	MMA (μmol/l)	tHcy (μmol/l)	FCA (%)	PCA (%)	PG A/C ratio	PG A (μg/l)	Gastrin (pmol/l)	Response to Cbl suppl.
Reference values			150–750	♂8.1–10.7 ♀7.3–9.7	80–98	<5	0	<0.32	<19.9	>19.7	>9.4	>1.6	>17	<70	
1. 76/F	Iron deficiency	Abdominal pain, vomiting	120	7.3	90	**6**	**2**	**0.43**	17.9	**8.2**	9.5	2.5	20	43	NA
2. 69/F	–	Cognitive decline	75	8.6	90	**14**	**1**	**0.90**	**22.5**	**9.5**	**5.2**	**0.4**	**6**	**780**	3, 4
3. 90/M	–	Attempted suicide	130	**6.0**	77	**13**	**3**	0.20	**20.0**	**13.8**	**8.7**	1.6	67	14	4
4. 70/M	–	Cognitive decline	77	8.8	96	5	0	**0.75**	**27.8**	–	12.8	**0.8**	35	**1070**	1, 2, 4
5. 66/F	Achlorhydria	Paraesthesia	65	9.0	94	2	0	**0.43**	**21.0**	**18.4**	**5.3**	**0.1**	**1**	**309**	4
6. 73/F	–	Fatigue	48	9.0	**104**	**7**	**1**	**1.44**	**42.7**	**6.5**	**1.0**	**0.3**	**2**	**660**	2, 3, 4
7. 73/F	Cervical cancer, pelvic radiation	Depression, anxiety	66	8.0	94	**17**	**3**	**0.56**	**38.4**	**1.9**	**1.6**	2.8	153	21	2, 4
8. 79/F	–	Fatigue	37	7.6	**119**	**40**	**13**	**0.83**	**69.0**	**10.7**	**2.7**	**0.5**	**11**	**88**	2, 4
9. 77/M	–	Orthostatic hypotension with collapse	110	8.6	94	**13**	0	**0.41**	**22.0**	**15.8**	**4.1**	**0.7**	27	**201**	NA
10. 74/F	–	Delirium, stroke, urosepsis	120	7.9	**101**	**13**	**1**	**0.39**	**30.0**	36.7	15.9	3.7	37	19	NA
11. 72/F	–	Diarrhoea	100	8.6	91	3	**1**	0.24	12.6	**14.0**	**8.1**	3.7	37	21	1, 3
12. 84/M	–	Cognitive decline	77	9.1	98	3	0	**0.46**	**24.7**	19.9	**5.9**	2.3	36	15	4
13. 68/F	Crohn's disease	Headaches	48	**7.0**	**100**	**14**	**5**	0.16	12.1	**4.0**	**1.4**	3.1	62	17	1
14. 72/F	–	Paraesthesia	100	8.1	89	**18**	**2**	**2.12**	13.8	**12.6**	**6.9**	**0.3**	**10**	**600**	4
15. 77/M	Folate deficiency	Previous low Cbl	76	**7.5**	94	**7**	0	**1.27**	**27.1**	26.0	**8.1**	2.5	160	51	NA
16. 79/M	–	Headaches	99	8.8	85	**11**	0	**0.42**	19.3	22.0	**4.4**	2.4	34	58	4
17. 81/F	–	Cognitive decline	69	8.0	92	**6**	0	**0.79**	19.0	**9.2**	**4.0**	**0.3**	**2**	24	NA
18. 68/F	Graves' disease	Esophagitis	110	8.3	91	4	0	**0.33**	**21.0**	22.1	12.6	4.2	105	29	NA
19. 78/M	–	Cognitive decline, gait problems	59	9.5	90	3	0	**0.53**	**22.0**	22.5	**7.5**	**0.6**	**4**	**73**	4
20. 75/M	–	Follow-up	76	8.2	93	3	0	**0.35**	**21.5**	37.6	**8.7**	3.6	61	24	NA
21. 85/M	–	Myelopathy	150	10.0	**101**	**9**	0	**0.59**	**25.5**	26.4	**8.2**	2.9	107	29	2, 4
22. 72/F	–	Check up	130	8.7	85	–	–	0.28	14.4	26.8	11.4	**0.3**	**6**	**200**	NA
23. 77/M	Partial gastrectomy	Depression	140	9.1	96	**15**	**1**	**0.53**	**25.3**	20.6	**6.0**	**0.5**	**13**	10	NA
24. 73/F	Partial gastrectomy	Dizziness, gait problems	96	8.9	95	**12**	0	**0.40**	17.2	**16.6**	**3.2**	**0.3**	**8**	18	2, 4
25. 79/F	–	Stroke	81	7.7	92	**15**	**1**	**0.36**	**21.5**	**11.9**	11.6	3.5	28	30	4
26. 66/F	–	Collapse e.c.i.	130	8.2	85	**11**	**1**	0.23	14.0	30.4	**8.0**	**1.3**	42	36	NA
27. 67/F	Partial gastrectomy	Depression	120	7.7	85	**15**	**2**	**0.47**	**20.9**	28.8	10.1	**0.6**	18	13	3, 4
28. 74/F	–	Gait problems	110	8.2	96	**19**	**2**	**1.62**	19.4	**3.1**	**1.8**	**0.3**	**4**	**1800**	2, 4

[a] Cbl, cobalamin; Hb, hemoglobin; MCV, mean cell volume; neutro's, neutrophils; MMA, methylmalonic acid; tHcy, total homocysteine; FCA, free cobalamin absorption; PCA, protein-bound cobalamin absorption; PG, pepsinogen; NA, not available. Response to Cbl suppl.: 1, increase in hemoglobin concentration of ≥0.5 mmol/l; 2, decrease of MCV of ≥5 fl; 3, clearing of hypersegmented neutrophils; 4, normalization of plasma MMA or tHcy. Abnormal values in bold type.

Response to Cbl supplementation

All 18 patients available for follow-up showed one or more responses to Cbl supplementation (Table 3). The following responses were observed: (1) an increase in haemoglobin concentration of 0.5 mmol/l in three (cases 4, 11 and 13); (2) a decrease in mean cell volume of ≥5 fl in seven (4, 6, 7, 8, 21, 24 and 28); (3) a clearing of hypersegmentation in three (2, 6 and 11); (4) a normalisation of MMA and/or tHcy in all 16 patients with elevated pre-treatment levels. Haemoglobin level and MCV did not change sig-

Table 3
Haematological and metabolic responses to cobalamin supplementation[a]

	Before ($n = 18$)	After ($n = 18$)	P
Hemoglobin (mmol/l)	8.6 (7.7–9.0)	8.6 (8.0–9.3)	0.212
Mean cell volume (fl)	94 (90–99)	90 (88–92)	0.079
Five-lobed neutrophils/100 neutrophils	15 (9–16)[b]	7 (5–10)	0.004
Six-lobed neutrophils/100 neutrophils	1 (0–2)[b]	0 (0–0)	0.008
Plasma methylmalonic acid (μmol/l)	0.50 (0.39–0.85)	0.14 (0.11–0.16)	<0.001
Plasma total homocysteine (μmol/l)	21.3 (18.8–26.1)	13.5 (10.2–17.1)	<0.001

[a] Results presented as median (25th and 75th percentile).
[b] Fourteen blood smears available.

nificantly after Cbl therapy. Anaemia was corrected in one patient (1/1) and macrocytosis was corrected in three patients (3/3).

Discussion

We investigated whether older patients with low plasma Cbl levels are Cbl deficient. We considered Cbl deficiency to be present when at least one of the following criteria was fulfilled: (1) the presence of haematological or metabolic abnormalities (anaemia, macrocytosis, neutrophilic hypersegmentation, elevated plasma MMA or tHcy levels), not attributable to other clinical conditions; (2) the presence of Cbl malabsorption or associated gastric abnormality, i.e. atrophic gastritis; (3) the presence of a haematological or metabolic response to Cbl supplementation. In this study, all investigated older patients with low plasma Cbl levels were Cbl deficient: 18 patients (64%) fulfilled all three criteria of Cbl deficiency, eight patients (29%) fulfilled two criteria and two patients (7%) fulfilled one criterion. The diagnostic myriad of tests applied in this study were used to investigate the hypothesis that low plasma Cbl, contrary to a number of reports in the literature [2–5], is a clinically significant finding consistent with the diagnosis of Cbl deficiency.

A limitation of the present study is that only a minority of the 128 patients with low Cbl levels participated in the study: 40% were excluded based on predefined criteria, while 63% of the remaining eligible patients declined participation. Patient recruitment is a well recognized and major problem in clinical studies with older patients [20]. Most patients had to be excluded because they could not comply with the protocol for urine collection which was necessary for the Cbl absorption tests. We have no indications that the 28 patients were not representative of the entire group with low Cbl levels. However, we cannot exclude this possibility.

We will now discuss the relative importance of the different haematological, metabolic, gastrointestinal and therapeutic findings of our study with regard to the diagnosis of Cbl deficiency.

Although anaemia or macrocytosis were absent in the majority, most patients had neutrophilic hypersegmentation. However, mild hypersegmentation as in our patients is not looked for or reported on by laboratory personnel. As mentioned before, hypersegmentation should be requested specifically [7]. Neutrophilic hypersegmentation is an early and sensitive feature of a megaloblastic state [21]. Recently, its value in the diagnosis of mild Cbl deficiency has been questioned [22]. In this study, the number of five-lobed neutrophils decreased significantly after Cbl supplementation, but remained above 5% in most patients. However, the number of six-lobed neutrophils declined significantly after Cbl therapy and cleared in almost every patient. Therefore, the presence of six-lobed neutrophils appears to be a sensitive diagnostic tool of mild Cbl deficiency, at least in older patients [23].

Plasma levels of MMA and tHcy have proven to be highly sensitive indicators of tissue cobalamin deficiency [7]. In the present study, the majority of patients (85.7%) had elevated plasma metabolite levels, which could not be ascribed to conditions other than Cbl deficiency such as renal failure or folate deficiency. In addition, the metabolite levels

normalised in almost every treated patient. Only four patients had normal plasma metabolites. However, all of them had other abnormalities of Cbl deficiency.

Cbl malabsorption was the most common cause of mild Cbl deficiency in this study. Defining the cause does not directly answer the question of whether a patient is Cbl deficient or not. In practice, however, documentation of the cause helps to support the diagnosis, especially when the clinical manifestations are equivocal. Therefore, measuring Cbl absorption, although difficult to perform in a number of elderly as the results are totally dependent on accurate urine collection, is generally considered as an important diagnostic tool. Moreover, since most causes of Cbl malabsorption are irreversible, the diagnosis of Cbl malabsorption warrants lifelong Cbl supplementation. Nearly half of the patients had normal Schilling test results. Most of them had food Cbl malabsorption as indicated by abnormal protein-bound Cbl absorption tests. This shows that a normal Schilling test does not exclude Cbl malabsorption. Because the classical Cbl malabsorption syndromes are also identified with the protein-bound Cbl absorption test, we propose this test as the method of first choice to diagnose Cbl malabsorption in older patients.

The reported prevalence of food Cbl malabsorption in older Cbl-deficient patients ranges from 8% [24] to 35.7% [25]. The prevalence of food Cbl malabsorption in our study was 28.6%. Food Cbl malabsorption has been described in patients with compromised gastric function, however the relative importance of acid-pepsin digestion for the release of protein-bound Cbl remains to be established. It also arises from as yet unknown mechanisms [26]. Indeed, most of our patients with food Cbl malabsorption had normal plasma pepsinogens levels.

Food Cbl malabsorption does not seem to result from mere age-associated changes. Previously, we found that healthy older subjects absorb protein-bound Cbl normally [11]. In addition, Cbl absorption is not a transient phenomenon [8,27] and can even evolve into pernicious anaemia [28,29]. Furthermore, in this study, food Cbl malabsorption caused metabolic abnormalities no different from the classical Cbl malabsorption states such as pernicious anaemia. Therefore, life-long supplementation of cobalamin in the case of food Cbl malabsorption should be warranted.

In this study the presence and severity of atrophic gastritis was assessed indirectly by measurements of plasma pepsinogens and gastrin. The determination of the ratio of pepsinogen A to C has been described as a valid diagnostic substitute for a biopsy of the gastric mucosa [30]. In this study we found atrophic gastritis (mostly severe) in nearly half of the patients with low Cbl levels, confirming earlier reports on the high prevalence of atrophic gastritis in the elderly [31,32].

All our patients showed haematological or metabolic responses to Cbl supplementation. To define a hematological response we adopted criteria from Stabler et al. [33]. The fact that some patients with haematological responses had no anemia or macrocytosis is thought to indicate the presence of subclinical abnormalities, especially since impaired DNA synthesis in bone marrow cells is demonstrated in 50% of elderly cobalamin-deficient patients who have no anemia or macrocytosis [34]. The relatively modest responses are not surprising given the mildness of the initial abnormalities. Nonetheless, the increase in haemoglobin level, the decrease in mean cell volume, the clearing of six-lobed neutrophils and the normalisation of elevated metabolite levels all strongly support the diagnosis of Cbl deficiency.

Although the data suggest that older hospital patients with low Cbl levels are Cbl deficient, the opposite may not be true, i.e. normal Cbl levels do not exclude Cbl deficiency. Some older people with normal Cbl levels may also have elevated plasma metabolite levels [35,36]. However, the clinical significance is still unclear.

In conclusion, we suggest that older patients with low Cbl levels are Cbl deficient. The majority of patients do not have abnormalities that are apparent upon first look, but require an in-depth investigation. This study shows that there is no single test that can be used as a gold standard. Instead, it is the constellation of haematological, metabolic and gastrointestinal findings that define the deficiency. If left untreated, progression of the deficiency seems inevitable because malabsorption of Cbl will persist and tissues will become increasingly Cbl deplete. Early treatment is important in preventing permanent (neurological) damage [37,38]. Furthermore, the

consequences of mild Cbl deficiency extend beyond its relevance for the haematological or neurological domains. For example, low Cbl levels are associated with impaired humoral immunity [39], and elevated plasma tHcy levels are now recognized as an increased risk for cardiovascular disease [40]. These findings underline that older patients with low Cbl levels should receive lifelong Cbl treatment.

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