Prevalence and haemopoietic effects of low serum vitamin B_{12} levels in geriatric medical patients

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The clinical significance of low serum vitamin B_{12} levels in elderly people is controversial. We aimed to document the prevalence of a low serum vitamin B_{12} (< 175 pmol/l) in patients referred to a geriatric medical unit, and to determine whether haemopoiesis is commonly affected in elderly patients with low serum vitamin B_{12}. We studied prospectively 472 consecutive referrals to a geriatric medical unit; fifty-six (13 %) had a low serum vitamin B_{12} level, of whom nineteen (34 %) of the fifty-six also had evidence of Fe deficiency (serum ferritin < 45 ng/ml). Low vitamin B_{12} was associated with a raised mean erythrocyte volume (MCV; mean 96·0 (SD 6·7) fl), compared with a control group (91·7 (SD 6·0) fl; $P = 0·001$). However, only thirteen (23 %) of the fifty-six patients with a low vitamin B_{12} had an MCV ≥ 100 fl. Mean haemoglobin (Hb) levels were not significantly reduced in those with a low vitamin B_{12}. In a subsequent study the haematological response to intramuscular hydroxocobalamin was examined in thirty-four patients with a low serum vitamin B_{12}. Treatment resulted in a significant fall in MCV and rise in Hb; these effects could be detected both in those patients with an initially normal full blood count (change in MCV −1·2 (SD 1·2); Hb + 0·5 (SD 0·6); $P < 0·01$) and in those with macrocytosis and/or anaemia (−9·1 (SD 11·8); + 0·8 (SD 1·2); $P < 0·05$). A low serum vitamin B_{12} is common in geriatric medical patients. This is usually associated with an upset in erythropoiesis, although the abnormalities are often subtle and may not be apparent on inspection of the full blood count. Elderly patients with serum vitamin B_{12} < 175 pmol/l should be assumed to have vitamin deficiency even if their full blood count is normal.

Elderly: Vitamin B_{12}: Erythrocytes

Serum vitamin B_{12} levels gradually decrease with ageing, (Mattila *et al.* 1986; Basun *et al.* 1994; Lindenbaum *et al.* 1994), and it is likely that the prevalence of vitamin B_{12} deficiency is increased in the elderly. Vitamin B_{12} deficiency can cause numerous clinically important adverse effects, including megaloblastic anaemia, neuropsychiatric disorders such as dementia, psychosis, peripheral neuropathy, and subacute combined degeneration of the spinal cord, and gastrointestinal problems such as glossitis and malabsorption (Boddy *et al.* 1972; Savage *et al.* 1995). The threshold level of serum vitamin B_{12} below which clinically important deficiency is likely to exist is controversial. Patients with pernicious anaemia usually have a level < 90 pmol/l (Anderson, 1964) and a megaloblastic marrow is commonly found in patients with a vitamin B_{12} level < 115 pmol/l (Boddy *et al.* 1972). Nearly all those with neuropsychiatric disorder thought to be due to vitamin B_{12} deficiency have a level < 175 pmol/l (Lindenbaum *et al.* 1988); these patients may have a normal full blood count. Metabolic evidence of vitamin B_{12} deficiency, including elevated

plasma levels of methylmalonic acid, can be shown with serum vitamin B_{12} levels of up to 258 pmol/l (Pennypacker *et al.* 1992). However, the clinical importance of these metabolic alterations is uncertain as they are frequently not associated with obvious haematological or clinical evidence of vitamin B_{12} deficiency. For the purposes of the present study we chose to define a low serum vitamin B_{12} level as < 175 pmol/l, consistent with the previously described clinical studies. This value is used as the lower end of the reference range by many haematology laboratories.

We aimed to document the prevalence of a low serum vitamin B_{12} (< 175 pmol/l) in patients referred to our geriatric medical unit, and to determine whether haemopoiesis is affected in elderly patients with low serum vitamin B_{12}. Data from the present study have been presented to the British Geriatrics Society and published in an abstract (Langhorne *et al.* 1992).

METHODS

We studied prospectively 472 consecutive new referrals to a geriatric medical unit. They comprised 330 in-patients, and 142 out-patients or day hospital attenders. There were 333 women (mean age 82 (range 63–101) years) and 139 men (mean age 79 (range 62–94) years). We have published other data from this patient group on the value of plasma ferritin (Holyoake *et al.* 1993) and the erythrogram (McKay *et al.* 1993) in the diagnosis of Fe deficiency.

As part of the standard assessment protocol for the unit a venous blood sample was taken from all patients for full blood count (FBC; Coulter S Plus IV; Coulter Electronics Ltd, Luton, Beds.), serum vitamin B_{12}, erythrocyte folate (radiosorbent assays; Becton Dickinson UK Ltd, Cowley, Oxford), and plasma ferritin (immunoradiometric assay; Ciba-Corning; Chiron Diagnostics, Halstead, Essex). Patients found to have malignancy (*n* 39), inflammatory joint disease (Casale *et al.* 1981), other connective tissue disease (Boddy *et al.* 1972), or chronic sepsis (Hoffbrand *et al.* 1966) were coded as having chronic inflammatory disease (*n* 67; two patients each had two conditions coded). A serum ferritin ≤ 45 ng/ml was accepted as evidence of Fe deficiency. Serum ferritin rises with ageing (Casale *et al.* 1981), and the threshold level for diagnosis of probable Fe deficiency is higher in elderly subjects than in young or middle-aged subjects (Guyatt *et al.* 1990; Holyoake *et al.* 1993). An erythrocyte folate level of < 75 ng/ml was accepted as evidence of folic acid deficiency, consistent with studies of healthy populations and in megaloblastic anaemia (Hoffbrand *et al.* 1966).

Patients with serum vitamin $B_{12} > 175$ pmol/l, ferritin ≥ 100 ng/ml, erythrocyte folate ≥ 75 ng/ml, and no known chronic inflammatory disease (*n* 253) were used as a control group for comparison with patients with a low serum vitamin B_{12} (< 175 pmol/l).

In a separate study the haematological response to intramuscular vitamin B_{12} was assessed prospectively in thirty-four consecutive new referrals with vitamin $B_{12} < 175$ pmol/l. These comprised seven men and twenty-seven women; mean age was 82 years. Patients with serum ferritin ≤ 45 ng/ml (indicating probable Fe deficiency) or erythrocyte folate below the lower limit of the laboratory reference range (75 pmol/l) were excluded. Those in an unstable clinical condition were not enrolled into the study to avoid in particular the effects of changes in hydration (due to illness or changes in drug treatment) on haemoglobin concentration. Patients were treated with intramuscular hydroxocobalamin, 1 mg given on alternate days for 1 week (total of four doses). The full blood count was checked at baseline and at 4 weeks after treatment. Blood sampling was performed after at least 15 min sitting at rest, between 09.00 and 12.00 hours, to

minimize any confounding postural and diurnal effects on the full blood count. Patients who developed an intercurrent illness or had a change in drugs that could alter hydration (particularly diuretics) were excluded from analysis. This study comprised careful recording of usual clinical practice and so did not require ethical committee approval.

Comparison of patients with a low serum vitamin B_{12} with the control group were made using Student's unpaired *t* test (two-tailed). Haematological responses to intramuscular hydroxocobalmin were analysed using Student's paired *t* test (two-tailed). The relationship between mean cell volume (MCV) and serum vitamin B_{12} (for patients with a level < 175 pmol/l) was analysed using Pearson's correlation coefficient. Differences were accepted as statistically significant at $P < 0{\cdot}05$. Results are expressed as mean and 1 SD, except where otherwise stated.

RESULTS

In the initial survey, nineteen (4 %) of the 472 patients were already receiving intramuscular vitamin B_{12} and were excluded from further study; fifty-six (13 %) of the patients had a serum vitamin B_{12} level < 175 pmol/ml, of whom nineteen (34 %) also had evidence of Fe deficiency (serum ferritin ≤ 45 ng/ml). A low erythrocyte folate (< 75 ng/ml) was present in three of the 472 patients, one of whom also had a low serum vitamin B_{12}.

Subjects with a low serum vitamin B_{12} but normal ferritin and erythrocyte folate levels (*n* 37) had a raised MCV compared with the control group (unpaired Student's *t* test, $P = 0{\cdot}001$; Table 1), but only thirteen (23 %) of the fifty-six patients with a low vitamin B_{12} had an elevated MCV (≥ 100 fl). For those with a low serum vitamin B_{12} but normal ferritin and erythrocyte folate, there was a significant inverse correlation between MCV

Table 1. *Haemoglobin (Hb), mean cell volume (MCV) and erythrocyte distribution width (RDW) in patients with a low serum vitamin B_{12} compared with control subjects and patients with combined low serum vitamin B_{12} and iron deficiency, all referred to a geriatric medical unit*‡

(Mean values and standard deviations (1SD)

n...	Low serum vitamin B_{12} 37		Control subjects 253		Combined low serum vitamin B_{12} and Fe deficiency 19	
	Mean	1SD	Mean	1SD	Mean	1SD
Male:female	15:22		18:172		1:18	
Serum vitamin B_{12} levels (pmol/l)	< 175		≥ 175		< 175	
Serum ferritin (ng/ml)	> 45		≥ 100		≤ 45	
Hb (g/l):						
Males	140	12	137	22	–	
Females	128	10	129	18	114†	24
MCV (fl)	96·0***	6·7	91·7	6·0	88·7††	10·0
RDW (%)	14·1	1·1	14·3	1·8	15·3†	1·8

Mean value was significantly different from that for control subjects (unpaired Student's *t* test): *** $P < 0{\cdot}001$.
Mean values were significantly different from those for subjects with a low serum vitamin B_{12} (unpaired Student's *t* test): † $P < 0{\cdot}05$, †† $P < 0{\cdot}01$.
‡ For details of subjects and procedures, see p. 58–59.

Table 2. *Prospective study of the haematological response to intramuscular hydroxocobalamin treatment of geriatric medical patients*†

(Mean values and standard deviations)

n...	Normal FBC 14		Abnormal FBC 12		Statistical significance of difference between groups‡: *P*<
	Mean	SD	Mean	SD	
Baseline data					
Age (years)	82	7	81	7	NS
Female:male	12:2		9:3		NS
Haemoglobin (g/l)	131	13	107	28	0·05
MCV (fl)	92	3·5	108	15	0·01
Serum vitamin B_{12} level (pg/ml)	136	29	107	35	0·05
Time between diagnosis and the last normal vitamin B_{12} level recorded (months)	11§	9	43§	30	0·01
Anti-parietal cell antibodies	2/9‖		4/10‖		NS
Anti-intrinsic factor antibodies	1/9‖		2/10‖		NS
Response to hydroxocobalamin treatment					
Change in MCV (fl)	−1·2**	1·2	−9·1*	11·8	
Change in haemoglobin (g/l)	+5**	6	+8*	12	

FBC, full blood count; MCV, mean cell volume.

Mean values for changes from baseline were significant (paired Student's *t* test): * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

† For details of subjects and procedures, see p. 58–59.

‡ The mean values for normal FBC and abnormal FBC groups were compared using unpaired Student's *t* test or Fisher's exact test.

§ Mean values for eight and ten subjects respectively for normal FBC and abnormal FBC groups.

‖ Values represent the number of patients with detectable antibodies relative to the number of patients in whom antibodies were sought.

and vitamin B_{12} level (*n* 37, r −0·51, F 12·2, $P = 0{\cdot}0013$). Mean haemoglobin (Hb) levels were not significantly reduced in those with a low serum vitamin B_{12}. Of fifty-six such patients, only eleven were anaemic (males Hb < 125 g/l, females < 116 g/l), of whom eight had co-existent Fe deficiency (ferritin ≤ 45 ng/ml).

Of the thirty-four recruited to the prospective study of haematological response to intramuscular hydroxocobalamin, five patients had to be withdrawn due to intercurrent illness, and three were lost to follow-up, leaving a total of twenty-six who completed the study. They were split into two groups for analysis; patients with macrocytosis (MCV ≥ 100 fl) and/or anaemia (males Hb < 125 g/l, females < 116 g/l; *n* 12), and those with a normal full blood count (*n* 14; Table 2). Patients who had an initially normal full blood count had higher vitamin B_{12} levels at diagnosis than those with macrocytosis and/or anaemia (unpaired Student's *t* test, $P < 0{\cdot}05$) and a shorter duration between diagnosis and the last normal vitamin B_{12} level recorded ($P < 0{\cdot}01$). Vitamin B_{12} replacement treatment resulted in a significant fall in MCV and rise in Hb; these effects could be detected both in those patients with an initially normal full blood count and in those with macrocytosis and/or anaemia (Table 2).

DISCUSSION

We found a previously unrecognized low serum vitamin B_{12} (< 175 pmol/ml) in 13 % of consecutive referrals to a geriatric medical unit. A low vitamin B_{12} was associated with a modestly elevated MCV, which invariably decreased after treatment with intramuscular

hydroxocobalamin. Low serum vitamin B_{12} levels in elderly patients have previously been reported to be associated with an abnormal bone marrow deoxyuridine suppression test (Carmel & Karnaze, 1985; Carmel *et al.* 1987*a*) and elevated serum methylmalonic acid and homocysteine (Pennypacker *et al.* 1992). Our results give more direct evidence that a serum vitamin B_{12} level of < 175 pmol/ml indicates deficiency of this vitamin. Metabolic evidence of vitamin B_{12} deficiency, including elevated plasma levels of methylmalonic acid, can be shown with serum vitamin B_{12} levels of up to 258 pmol/l (Pennypacker *et al.* 1992). However, the clinical importance of these metabolic alterations is uncertain as they are frequently not associated with obvious haematological or clinical evidence of vitamin B_{12} deficiency.

Only 23 % of our patients with low serum vitamin B_{12} had overt macrocytosis (≥ 100 fl). Macrocytosis can be masked by co-existent Fe deficiency, which is reported in 20–25 % of patients with pernicious anaemia (Carmel *et al.* 1987*b*; Atrah & Davidson, 1988). We found evidence of Fe deficiency (ferritin ≤ 45 ng/ml) in 34 % of those with a low vitamin B_{12}. The majority of those that were anaemic had co-existent Fe deficiency. It is clear that elderly patients with a low vitamin B_{12} cannot be reliably identified by examination of the full blood count for macrocytosis.

Most elderly patients with a low serum vitamin B_{12} level have malabsorption of vitamin B_{12} due to either autoimmune pernicious anaemia with deficiency of intrinsic factor, or to hypo- or achlorhydria associated with atrophic gastritis (Logan *et al.* 1989). Atrophic gastritis is associated with poor absorption of protein-bound vitamin B_{12} (the form present in the diet) which is not corrected by intrinsic factor. However, absorption of unbound vitamin B_{12}, as administered in a standard Schilling test (Fairbanks, 1983) is usually normal (Logan *et al.* 1989). The prevalence of pernicious anaemia (Mosbech, 1952), of gastric hypo- or achlorhydria and of atrophic gastritis (Yelland, 1991) increase with ageing. Small bowel disease is thought to be a relatively rare cause of vitamin B_{12} deficiency in elderly patients (Logan *et al.* 1989), but should be considered when suggestive symptoms or other nutritional deficiencies are present. Another group of elderly people that may be more likely to develop vitamin B_{12} deficiency are those with low dietary intake, including dementia sufferers who remain in the community, who have lower serum vitamin B_{12} levels than those in institutional care (Basun *et al.* 1994).

The rise in MCV and megaloblastic haematopoiesis associated with vitamin B_{12} deficiency is secondary to impaired DNA synthesis (Tefferi & Pruthi, 1994). Two main mechanisms have been proposed, the methylfolate trap hypothesis where dietary folate is inaccessible for polyglutamation, and the formate starvation hypothesis with failure to use already polyglutamated forms of folate (Tefferi & Pruthi, 1994).

Neuropsychiatric complications of vitamin B_{12} deficiency can occur when haemopoiesis is mildly upset or unaffected (Martin, 1988). However, the proportion of patients with low serum vitamin B_{12} who develop such complications is unclear. One study of neuropsychiatrically-impaired patients with low serum vitamin B_{12} reported that vitamin replacement was invariably followed by neuropsychiatric improvement (Lindenbaum *et al.* 1988). However, this study was uncontrolled with open (unblinded) patient assessment. Abnormal somato-sensory evoked potentials have been described in a small group of patients with low serum vitamin B_{12} levels, but the lack of an adequate control group raises questions about the validity of these findings (Karnaze & Carmel, 1990). Serum vitamin B_{12} tends to be reduced in patients with dementia compared with healthy controls (Kristensen *et al.* 1993), but the clinical importance of this is often uncertain. In a randomized, placebo-controlled, parallel group study of the effects of intramuscular vitamin B_{12} in elderly patients with low serum vitamin B_{12}, treatment caused no significant

change in haemoglobin or in psychiatric symptoms such as anxiety or lethargy (Hughes *et al.* 1970). However, this study did not look at changes in erythrocyte indices or detailed neuropsychiatric function. Even if established neurological or psychiatric effects of vitamin B_{12} deficiency were irreversible, replacement therapy might prevent their progression.

Vitamin B_{12} deficiency may also be a risk factor for stroke, myocardial infarction, and peripheral arterial disease, by causing hyperhomocysteinaemia (Kang *et al.* 1992). It is claimed that this amino acid causes atherothrombosis through a variety of mechanisms. Homocysteine is toxic to vascular endothelium (Harker *et al.* 1976), can potentiate the auto-oxidation of LDL-cholesterol (resulting in a highly atherogenic lipid profile; Heinecke *et al.* 1987), and promotes thrombosis by enhancing platelet aggregation (Harker *et al.* 1987) or inhibition of activated protein C (Rogers & Conn, 1995).

We have shown that low serum vitamin B_{12} levels (< 175 pmol/ml) in elderly patients are common and are associated with reversible changes in erythropoiesis, indicating tissue deficiency. These results lend support to the clinical practice of routine vitamin B_{12} replacement treatment for patients found to have a vitamin B_{12} levels of < 175 pmol/ml. Further studies are required to determine whether higher threshold levels of serum vitamin B_{12} warrant treatment, and whether vitamin B_{12} replacement can prevent or ameliorate common neuropsychiatric illnesses or atherothrombotic vascular disease.

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