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 haemoopoiesis 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 serum 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\textsubscript{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\textsubscript{12} deficiency. For the purposes of the present study we chose to define a low serum vitamin B\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{12} (< 175 pmol/l).

In a separate study the haematological response to intramuscular vitamin B\textsubscript{12} was assessed prospectively in thirty-four consecutive new referrals with vitamin B\textsubscript{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 B₁₂ with the control group were made using Student’s unpaired t test (two-tailed). Haematological responses to intramuscular hydroxocobalamin were analysed using Student’s paired t test (two-tailed). The relationship between mean cell volume (MCV) and serum vitamin B₁₂ (for patients with a level <175 pmol/l) was analysed using Pearson’s correlation coefficient. Differences were accepted as statistically significant at \( P < 0.05 \). 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 B₁₂ and were excluded from further study; fifty-six (13%) of the patients had a serum vitamin B₁₂ 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 B₁₂.

Subjects with a low serum vitamin B₁₂ but normal ferritin and erythrocyte folate levels (n=37) had a raised MCV compared with the control group (unpaired Student’s t test, \( P=0.001 \); Table 1), but only thirteen (23%) of the fifty-six patients with a low vitamin B₁₂ had an elevated MCV (≥100 fl). For those with a low serum vitamin B₁₂ 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 B₁₂ compared with control subjects and patients with combined low serum vitamin B₁₂ and iron deficiency, all referred to a geriatric medical unit‡

<table>
<thead>
<tr>
<th></th>
<th>Low serum vitamin B₁₂</th>
<th>Control subjects</th>
<th>Combined low serum vitamin B₁₂ and Fe deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=37</td>
<td>253</td>
<td>19</td>
</tr>
<tr>
<td>Male:female</td>
<td>Mean 1SD</td>
<td>Mean 1SD</td>
<td>Mean 1SD</td>
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<tr>
<td>Serum vitamin B₁₂ levels (pmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;175</td>
<td>15:22 96.0*** 140</td>
<td>18:172 91.7 128</td>
<td>1:18</td>
</tr>
<tr>
<td>≥175</td>
<td></td>
<td>≥175 137 129</td>
<td>&lt;175</td>
</tr>
<tr>
<td>Serum ferritin (ng/ml)</td>
<td></td>
<td></td>
<td>≥100</td>
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<tr>
<td>&gt;45</td>
<td>14:1</td>
<td>≥45 137 129</td>
<td>≤45</td>
</tr>
<tr>
<td>Hb (g/l):</td>
<td>Males</td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>140 96.0*** 128</td>
<td>128</td>
<td>114† 114† 24</td>
</tr>
<tr>
<td></td>
<td>6-7</td>
<td>10 18</td>
<td>4-0 10-0</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>1-1</td>
<td>1-1</td>
<td>1-8 1-8</td>
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</table>

Mean value was significantly different from that for control subjects (unpaired Student’s t test): *** \( P < 0.001 \).
Mean values were significantly different from those for subjects with a low serum vitamin B₁₂ (unpaired Student’s t test): † \( P < 0.05 \), †† \( P < 0.01 \).
‡ 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)

<table>
<thead>
<tr>
<th>Statistical significance of difference between groups‡: P&lt;</th>
</tr>
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<tbody>
<tr>
<td>n...</td>
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<tr>
<td></td>
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<tr>
<td>Baseline data</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Female:male</td>
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<tr>
<td>Haemoglobin (g/l)</td>
</tr>
<tr>
<td>MCV (fl)</td>
</tr>
<tr>
<td>Serum vitamin B₁₂ level (pg/ml)</td>
</tr>
<tr>
<td>Time between diagnosis and the last normal vitamin B₁₂ level recorded (months)</td>
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<tr>
<td>Anti-parietal cell antibodies</td>
</tr>
<tr>
<td>Anti-intrinsic factor antibodies</td>
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<tr>
<td>Response to hydroxocobalamin treatment</td>
</tr>
<tr>
<td>Change in MCV (fl)</td>
</tr>
<tr>
<td>Change in haemoglobin (g/l)</td>
</tr>
</tbody>
</table>

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

Mean values for changes from baseline were significant (paired Student’s t test): *P<0.05, **P<0.01.
† 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 B₁₂ level (n 37, r −0.51, F 12.2, P = 0.0013). Mean haemoglobin (Hb) levels were not significantly reduced in those with a low serum vitamin B₁₂. 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 B₁₂ levels at diagnosis than those with macrocytosis and/or anaemia (unpaired Student’s t test, P < 0.05) and a shorter duration between diagnosis and the last normal vitamin B₁₂ level recorded (P < 0.01). Vitamin B₁₂ 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 B₁₂ (<175 pmol/ml) in 13% of consecutive referrals to a geriatric medical unit. A low vitamin B₁₂ was associated with a modestly elevated MCV, which invariably decreased after treatment with intramuscular
hydroxocobalamin. Low serum vitamin B₁₂ levels in elderly patients have previously been reported to be associated with an abnormal bone marrow deoxuridine suppression test (Carmel & Karnaze, 1985; Carmel et al. 1987a) and elevated serum methylmalonic acid and homocysteine (Pennypacker et al. 1992). Our results give more direct evidence that a serum vitamin B₁₂ level of < 175 pmol/ml indicates deficiency of this vitamin. Metabolic evidence of vitamin B₁₂ deficiency, including elevated plasma levels of methylmalonic acid, can be shown with serum vitamin B₁₂ 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 B₁₂ deficiency.

Only 23% of our patients with low serum vitamin B₁₂ 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. 1987b; Atrah & Davidson, 1988). We found evidence of Fe deficiency (ferritin ≤ 45 ng/ml) in 34% of those with a low vitamin B₁₂. The majority of those that were anaemic had co-existent Fe deficiency. It is clear that elderly patients with a low vitamin B₁₂ cannot be reliably identified by examination of the full blood count for macrocytosis.

Most elderly patients with a low serum vitamin B₁₂ level have malabsorption of vitamin B₁₂ 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 B₁₂ (the form present in the diet) which is not corrected by intrinsic factor. However, absorption of unbound vitamin B₁₂, 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 B₁₂ 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 B₁₂ deficiency are those with low dietary intake, including dementia sufferers who remain in the community, who have lower serum vitamin B₁₂ levels than those in institutional care (Basun et al. 1994).

The rise in MCV and megaloblastic haematopoiesis associated with vitamin B₁₂ 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 B₁₂ deficiency can occur when haemopoiesis is mildly upset or unaffected (Martin, 1988). However, the proportion of patients with low serum vitamin B₁₂ who develop such complications is unclear. One study of neuropsychiatrically-impaired patients with low serum vitamin B₁₂ 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 B₁₂ levels, but the lack of an adequate control group raises questions about the validity of these findings (Karnaze & Carmel, 1990). Serum vitamin B₁₂ 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 B₁₂ in elderly patients with low serum vitamin B₁₂, 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 B₁₂ deficiency were irreversible, replacement therapy might prevent their progression.

Vitamin B₁₂ 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 B₁₂ 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 B₁₂ replacement treatment for patients found to have a vitamin B₁₂ levels of < 175 pmol/ml. Further studies are required to determine whether higher threshold levels of serum vitamin B₁₂ warrant treatment, and whether vitamin B₁₂ replacement can prevent or ameliorate common neuropsychiatric illnesses or atherothrombotic vascular disease.

REFERENCES


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