J Clin Pathol 1985;**38**:1179–1184

Importance of low serum vitamin B_{12} and red cell folate concentrations in elderly hospital inpatients

EL BLUNDELL,* JH MATTHEWS,* SM ALLEN, AM MIDDLETON, JE MORRIS, SN WICKRAMASINGHE*

**Department of Haematology, St Mary's Hospital Medical School, London, and the Department of Medicine for the Elderly, St Charles' Hospital, London*

SUMMARY To determine the functional importance of the low B_{12} and red cell folate concentrations repeatedly observed in the elderly 200 consecutive patients admitted to a geriatric unit were studied. Forty six of the patients had low serum concentrations of B_{12} (15), red cell folate (26), or both (five). Serum B_{12} and red cell folate concentrations correlated with mean cell volume, and serum B_{12} correlated with the neutrophil lobe count. Bone marrow deoxyuridine suppression was abnormal in 35% of the patients with low vitamin concentrations, but 55% of those with abnormal deoxyuridine suppression had morphologically normal bone marrow, and 73% had a normal mean cell volume. In patients with low vitamin values the deoxyuridine suppressed value correlated with the haemoglobin concentration and neutrophil lobe count. Thus synthesis of thymidylate was impaired by vitamin B_{12} or folate deficiency in at least 8% of newly admitted elderly patients, many of whom had normal blood counts despite the biochemical disturbance affecting haemopoiesis. A nutritionally depleted diet may have been responsible for many of the low vitamin values.

Many reports indicate that low serum and red cell folate concentrations and low serum B_{12} concentrations often occur in elderly patients.[1–8] The low folate concentrations have been attributed to a nutritional deficiency[4] and the low B_{12} concentrations to various causes, including folate deficiency, atrophic gastritis, latent pernicious anaemia, malabsorption, and nutritional vitamin B_{12} deficiency.[2 9] There is, however, some controversy over whether these low vitamin concentrations are necessarily associated with megaloblastic haemopoiesis and, therefore, whether they indicate a true deficiency of these vitamins in haemopoietic and other tissues. Abnormal Figlu excretion and megaloblastic haemopoiesis were repeatedly found in elderly subjects with low serum concentrations of folate in two studies[4 8] and in those with low serum concentrations of vitamin B_{12} in one other.[2] On the other hand, Batata *et al* did not find megaloblastic changes in bone marrow aspirates from 94 patients despite low serum folate concentrations in 10 and low serum B_{12} in three,[1] and in another study of 39 patients, in whom the mean serum folate was low, there was judged to be "little evidence of folate deficiency".[3] More recently, Magnus *et al* found that hypersegmented neutrophils could be observed only when the serum B_{12}, the serum folate, and the red cell folate concentrations were all reduced and inferred that isolated low values of either B_{12} or folate were not synonymous with functional deficiency.[5] Furthermore, although it is widely held that the finding of a low serum B_{12} or red cell folate concentration is unimportant in the absence of macrocytosis, there is at present no objective evidence to support this view. We therefore set out to determine the prevalence of low serum B_{12} and red cell folate concentrations among newly admitted elderly patients and to determine their significance by performing deoxyuridine suppression tests on bone marrow cells.

The deoxyuridine suppression test measures the efficiency of the methylation of deoxyuridylate to thymidylate, which depends on adequate intracellular concentrations of both 5,10-

Accepted for publication 1 July 1985

methylenetetrahydrofolate and methylcobalamin, and is considered to limit the rate of synthesis of DNA.[11] The deoxyuridine suppression test is, therefore, a useful method of detecting impairment of an important biochemical process dependent on vitamin B_{12} and folate within the haemopoietic cells themselves. The test may give abnormal results at an early stage of the development of vitamin B_{12} or folate deficiency before the manifestation of obvious haematological changes.

Patients and methods

Two hundred consecutive patients admitted to the unit for the care of the elderly at St Charles' Hospital, London, were studied. The principal reasons for admission included failure to cope due to social, physical, or mental deterioration (44 patients); falls (42); chest infection (28); abdominal complaints (22); heart failure (20); stroke (14); leg ulcers (14); and others (16), although multiple disease was common. The social state of the subjects was noted: 116 patients lived alone; 64 with relatives, and 20 in institutions. Clinical details, treatment with drugs, and alcohol intake were recorded. During the first half of the study an abbreviated mental test score[10] (dementia score) and a neuropathy score were assessed either immediately before discharge, or two weeks after admission, whichever happened sooner. The neuropathy score was determined by allocating one point for the presence of each long tendon reflex and one point for the presence of vibration sensation at the ankle, giving a possible total of 12.

Haemoglobin concentration, mean cell volume, and serum B_{12}, serum and red cell folate, serum thyroxine, and serum albumin concentrations were measured and a neutrophil lobe count performed on admission. The haemoglobin concentration and mean cell volume were obtained from a Coulter Counter, model S, standardised with 4C cell control. The true serum B_{12} and the serum and red cell folate concentrations were determined using separate radioassay kits (Becton Dickinson; cobalt-57 and iodine-125, respectively); the vitamin B_{12} binding protein in the B_{12} radioassay kit was porcine intrinsic factor with the contaminating R proteins blocked by B_{12} analogues prepared by hydrolysis of vitamin B_{12}. With these kits the 95% reference intervals for the serum B_{12} and red cell folate concentrations were determined in our laboratory to be 165–684 ng/l (geometric mean 336 ng/l) and 200–800 μg/l (geometric mean 398 μg/l), respectively, for adults aged from 18 to 65 years.[11 12] The mean neutrophil lobe count was determined from a count of 100 neutrophils by a single observer using blood smears stained with May-Grünwald Giemsa.

Patients in whom either the serum B_{12} or red cell folate concentration was below the 95% reference range underwent bone marrow aspiration, when the measurement of serum B_{12} and red cell folate concentrations was repeated. Seven patients admitted to the same clinical unit with normal serum B_{12} and red cell folate concentrations also had bone marrow aspirated (elderly control patients); three from the study and one other needed a bone marrow examination for additional clinical purposes, and three were volunteers. Marrow smears were stained by May-Grünwald Giemsa and Perls's acid ferrocyanide methods and assessed by a single observer for the presence of megaloblasts and giant metamyelocytes and for the quantity of stainable iron in the fragments. A deoxyuridine suppression test as described by Wickramasinghe *et al* was carried out on all the marrow aspirates; the normal range for the deoxyuridine suppressed value obtained by this method was 1·4–8·6%.[13]

STATISTICS

Serum B_{12} and red cell folate values were subjected to logarithmic transformation before use in statisti-

Haematological details of patients studied (and 95% ranges)

	Men	*Women*	*Total*	*p**
No of patients	59	141	200	
Mean age (years)	78 (65·5–92·1)	82 (67·8–96·8)	81 (66·8–95·8)	< 0·001
Mean haemoglobin (g/l)	12·9 (9·0–16·8)	13·0 (8·9–17·0)	13·0 (9·1–16·9)	NS
Mean mean cell volume (fl)	91·6 (80·0–103·3)	88·7 (73·4–104·0)	89·6 (75·1–104·1)	0·005
Geometric mean serum B_{12} (ng/l)	374 (94–1510)	426 (106–1713)	410 (102–1651)	NS
Geometic mean red cell folate (μg/l)	276 (109–698)	351 (111–1105)	327 (108–988)	0·001
Mean serum albumin (g/l)	36·0 (26·2–45·8)	35·6 (26·6–44·5)	35·7 (26·5–44·6)	NS
Mean serum thyroxine (nmol/l)	93·9 (35·1–152·1)	96·1 (43·2–149·0)	95·6 (41·3–149·8)	NS

*For difference between sexes.
Conversion: SI to tradional units—Thyroxine: 1 nmol/l ≈ 77·7 ng/100 ml.

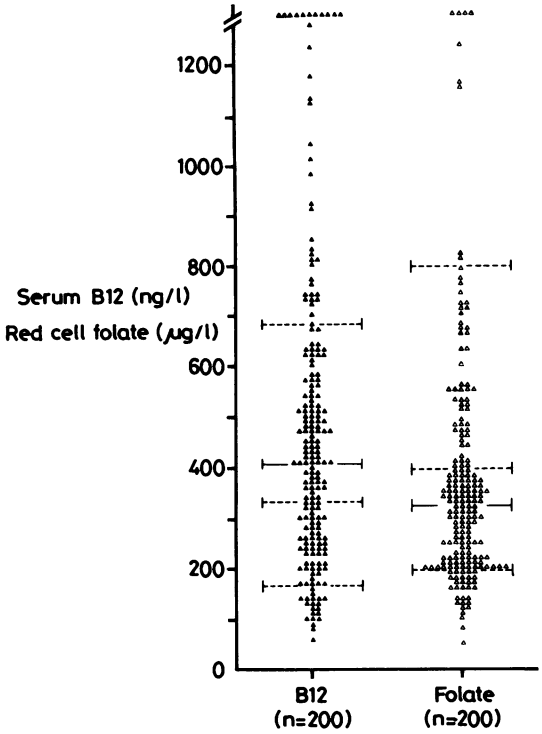

Fig. 1 *Serum vitamin B_{12} and red cell folate values in 200 patients. Solid bars indicate geometric mean. Dotted bars indicate normal geometric mean and 95% reference interval.*

cal analysis. When the populations were distributed normally Pearson's *r* and Student's *t* test were used, otherwise the Mann-Whitney U value and Spearman's R were calculated.

Results

The Table and Fig. 1 describe the 200 patients in terms of their age distribution, haematological values, and serum albumin and serum thyroxine concentrations. The mean serum B_{12} was significantly higher than the reference mean (410 *v* 336 ng/l; $p < 0{\cdot}002$), possibly because of undisclosed recent administration of vitamin B_{12} in some of the cases, and the mean red cell folate was significantly lower (327 *v* 398 µg/l; $p < 0{\cdot}001$). Serum B_{12} correlated with red cell folate concentrations ($R = 0{\cdot}3$; $p < 0{\cdot}001$), and with serum folate ($R = 0{\cdot}27$; $p = 0{\cdot}006$). Red cell folate correlated with serum folate ($R = 0{\cdot}63$; $p < 0{\cdot}001$). There was no correlation of serum albumin, dementia score and neuropathy score with either the serum B_{12} or the red cell folate concentrations which were also not influenced by social state. The mean cell volume showed a negative correlation with both serum B_{12} ($R = -0{\cdot}15$; $p = 0{\cdot}02$) and red cell folate ($R = -0{\cdot}21$; $p = 0{\cdot}001$). The neutrophil lobe count correlated negatively with serum B_{12} ($R = -0{\cdot}2$; $p = 0{\cdot}003$) but not with red cell folate.

Forty six patients had low concentrations of serum B_{12} (15), red cell folate (26), or both (5), and 20 of these were men, significantly more than expected ($p = 0{\cdot}04$ by χ^2 test). The mean cell volume in this group with low vitamin concentrations was higher than in the remaining 154 patients (92·6 *v* 88·7 fl; $p = 0{\cdot}003$), but exceeded 98 fl in only four cases. Only 15 of the patients with low vitamin concentrations were anaemic, and the mean haemoglobin concentration in the group with low vitamins was not lower than that in the other patients. Twenty five of the patients underwent a second assay of serum B_{12} and red cell folate at the time of the bone marrow aspirate, and a significant increase between the first and second values was observed in the B_{12} concentrations (Fig. 2; $p = 0{\cdot}002$ by the paired

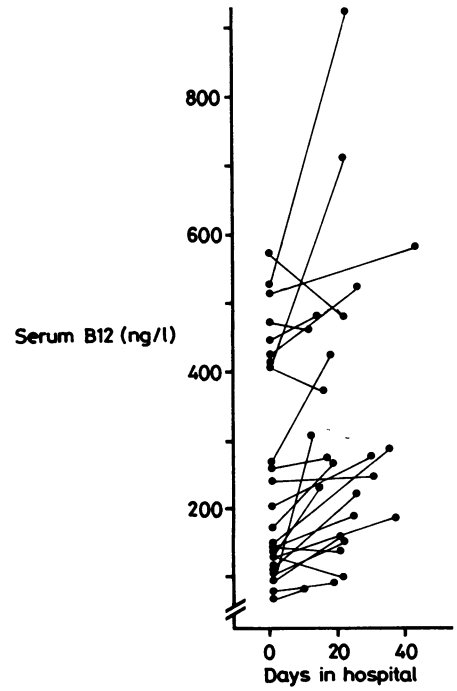

Fig. 2 *Serum vitamin B_{12} concentrations in 25 patients on admission and after a period in hospital. Second level is higher than first in 21 cases: $p = 0{\cdot}002$ by paired t test.*

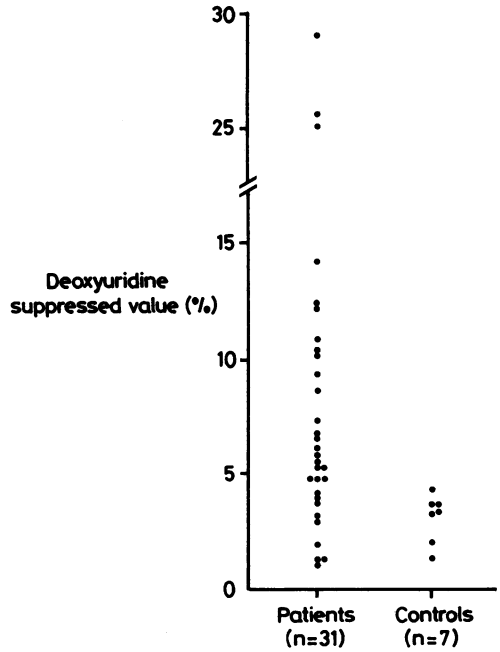

Fig. 3 *Deoxyuridine suppressed values in patients and elderly controls. (p for difference between means < 0·005).*

t test) but not the folate concentrations. The mean blood urea concentration was 8·6 mmol/l (51·8 mg/100 ml) among those with low vitamin values and 11·0 mmol/l (66·3 mg/100 ml) in the remainder (p = 0·03). There were no significant differences in age, dementia score, and serum albumin concentrations between those patients with and without low vitamin concentrations.

Marrow aspirates were taken from 31 of the 46 patients with low vitamin concentrations. The remaining 15 patients did not undergo marrow aspiration for various reasons, including death or discharge before the results of the assays were known, refusal, or unsuitability because of grave ill health.

Eleven of the marrow aspirates gave abnormal deoxyuridine suppressed values (Fig. 3); two of these showed megaloblastic erythropoiesis, and a further three contained giant metamyelocytes. In two patients haemopoiesis was megaloblastic, but the deoxyuridine suppressed value was normal. All of the patients had stainable iron in the marrow fragments. Of the patients with abnormal deoxyuridine suppressed values, four had low serum B_{12}, six had low red cell folate, and one had both values low at the time of admission. The mean haemoglobin concentration in the patients with abnormal deoxyuridine suppressed values was 11·5 g/dl (range 7·4–13·8 g/dl), and the mean cell volume was >98 fl in only two of them. In the 31 patients who had deoxyuridine suppression tests there was a negative correlation between haemoglobin concentration and deoxyuridine suppressed value (R = −0·55; p = 0·001), a positive correlation between deoxyuridine suppressed value and neutrophil lobe count (R = 0·33; p = 0·04), and a negative correlation between deoxyuridine suppressed value and serum albumin (R = −0·4; p = 0·03). Deoxyuridine suppressed values did not correlate with serum B_{12}, red cell folate, or mean cell volume and were not influenced by age, sex, dementia score, alcohol intake, the presence of infection, or serum thyroxine concentration. One patient giving an abnormal deoxyuridine suppressed value was taking phenytoin; one other patient taking phenytoin had a normal deoxyuridine suppressed value. Patients with abnormal deoxyuridine suppression were not taking co-trimoxazole or trimethoprim. With the addition of 50 μg folic acid/ml to the cultures with and without deoxyuridine the average deoxyuridine suppressed value (28 marrow samples) was reduced to 52% of the value without folic acid.

The marrow aspirates from seven elderly control patients contained morphologically normal haemopoietic cells and gave normal deoxyuridine suppressed values. The average deoxyuridine suppressed value given by these seven control patients was 3·2%, which was significantly lower than the corresponding figure of 8·4% given by the 31 patients with low serum B_{12} or red cell folate concentrations (p < 0·005).

Discussion

Our results indicate that many elderly patients with a low serum B_{12} or red cell folate concentration show an impairment of the methylation of deoxyuridylate to thymidylate in bone marrow cells, even when haemoglobin concentrations and mean cell volume are within the normal range. Such impairment of synthesis of thymidylate is known to occur in vitamin B_{12} or folate deficiency, in protein energy malnutrition, and is secondary to treatment with certain drugs.[13 14] High deoxyuridine suppressed values do not seem to be a part of the normal ageing process in view of the normal results observed in the elderly control patients. Only one patient with a high deoxyuridine suppressed value was taking

phenytoin, and patients were not taking any other drug known to cause an abnormality of thymidylate synthesis. Protein malnutrition could be directly affecting thymidylate synthesis, especially as a significant negative correlation between the deoxyuridine suppressed value and serum albumin concentration was observed. This correlation might, however, reflect only an association between a low serum albumin concentration and a subset of patients with particularly poor nutrition within the group with low B_{12} and folate concentrations. Moreover, the extent of the correction of the deoxyuridine suppressed value with the addition of folic acid was greater than that observed in children with protein energy malnutrition.[15] Thus we suggest that the observed impairment of thymidylate synthesis probably indicates true intracellular deficiency of vitamin B_{12} or folate, although some direct effect of protein malnutrition cannot be excluded.

Inadequate folate intake probably accounts for the high incidence of a low red cell folate concentration in the elderly.[4] Folate deficiency might be responsible for some of the low serum B_{12} values, but in 15 cases the low serum B_{12} was not associated with a low red cell folate. Latent pernicious anaemia is unlikely to account for a high proportion of the low serum B_{12} values; of four successful Schilling tests carried out in patients with an isolated low serum B_{12}, three yielded normal results. Possibly, therefore, some of the low serum B_{12} values were caused by nutritional deficiency of vitamin B_{12}. This view is supported by the finding that serum B_{12} values rose significantly after admission to hospital, where nutrition is likely to have improved. Rising serum B_{12} values were also found in one other study of institutionalised elderly patients, although after a longer period.[2] The increase in serum B_{12} in the period after admission to hospital also raises the possibility that the prevalence of abnormal deoxyuridine suppressed values might have been greater had our examination of the marrow been carried out earlier during the patients' admission.

The clinical importance of the low serum B_{12} and red cell folate values and abnormal deoxyuridine suppressed values in elderly patients remains to be determined. In most of our cases there was neither anaemia nor macrocytosis, and we found no evidence that dementia or neuropathy was associated with low serum B_{12} or red cell folate values, or with abnormal deoxyuridine suppressed values. On the other hand, there were significant negative correlations between mean cell volume and red cell folate, mean cell volume and serum B_{12}, haemoglobin concentration and deoxyuridine suppressed value, and neutrophil lobe count and serum B_{12} and a significant positive correlation between neutrophil lobe count and deoxyuridine suppressed value. Although these correlations were weak, possibly reflecting the interplay of several factors, taken together they suggest that the observed low vitamin concentrations have functional relevance. Furthermore, although bone marrow cells with partially impaired methylation of deoxyuridylate might be capable of sustaining steady-state haemopoiesis, they may be unable to meet increased demands in circumstances such as infection or haemolysis. In addition, as vitamin B_{12} and folate are required for the normal proliferation of cells other than those of the marrow, important functional defects may be occurring in various non-haemopoietic tissues and contributing to ill health. Possibly, treatment of elderly patients who have low B_{12} or folate concentrations with the appropriate vitamin might improve general health, haemoglobin concentration, and mean cell volume. This possibility has received inadequate attention so far and merits further investigation. Although one previous study showed that no clinical benefit accrued from treatment with B_{12} in terms of subjective wellbeing, psychiatric state, and haemoglobin concentration,[16] this was based on healthy elderly subjects residing in the community, and data on mean cell volume were not given.

We thank Mrs J Armitage for performing the deoxyuridine suppression tests and the staff of the computer unit for their help with the statistics.

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Request for reprints to: Dr JH Matthews, Department of Haematology, St Mary's Hospital Medical School, Norfolk Place, London W2 1PG, England.