

Vitamin B12 Deficiency—Need for a New Guideline

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OBJECTIVES: Many patients with vitamin B12 deficiency do not have anemia or macrocytosis, but the prevalence of B12 deficiency in patients without macrocytosis is not known.

METHODS: We investigated the prevalence of B12 deficiency among patients with normocytosis and microcytosis and recommended a screening strategy. All patients ($n = 3714$) with serum B12 measured at the Prince of Wales Hospital in 1996 were reviewed. The prevalence of serum B12 less than 140 pmol/L was determined for the following patient subgroups: younger than 70 y, older than 70 y, anemic, non-anemic, macrocytic, normocytic, microcytic, documented iron deficiency, and documented thalassemia.

RESULTS: The prevalence of B12 deficiency (<140 pmol/L) ranged from 4.8% to 9.8% among the different subgroups.

CONCLUSIONS: Whatever screening criteria were used, a significant number of B12-deficient patients will be missed. Therefore, there may be a case for universal vitamin B12 screening. *Nutrition* 2001;17:917–920. ©Elsevier Science Inc. 2001

KEYWORDS: vitamin B12, screening guidelines

INTRODUCTION

Vitamin B12 deficiency results in anemia, neurologic injury, and hyperhomocysteinemia and is a risk factor for coronary heart disease and stroke. The prevalence of vitamin B12 deficiency in the general population is not known and good screening guidelines are essential. Many patients with vitamin B12 deficiency have neither anemia nor macrocytosis.^{1–5} In one study, 81% of the 124 patients with B12 below 200 pg/mL had Mean Cell Volume (MCVs) below 95 fl.¹ Stott et al.² prospectively studied 472 consecutive geriatric referrals; 56 (13%) had low B12, but only 13 (23%) had MCVs above 100 fl. Luong et al.³ studied 59 anemic Vietnamese patients with low B12 or red blood cell folate levels. None had macrocytosis. MCV was normal in 40 and low in 19 patients. Therefore, some investigators have suggested that B12 and red blood cell folate levels should be determined in all anemic or geriatric patients, but there is no general consensus. Most studies first identified patients with B12 deficiency and then correlated the low B12 values with the MCV. There is no large study of the prevalence of B12 deficiency among patients with normocytosis or microcytosis. The reported prevalence in geriatric patients is 7.5% to 13%,^{4,5} but data on non-geriatric patients are lacking. Therefore, we retrospectively reviewed the clinical records of all 3741 patients with serum B12 measured at Prince of Wales Hospital in 1996. The prevalence of B12 deficiency was determined among the following patient subgroups: younger versus older than 70 y, anemic versus non-anemic patients, and macrocytosis versus normocytosis versus microcytosis. We discuss the impact of our observations on vitamin B12 screening.

MATERIALS AND METHODS

Setting

The Prince of Wales Hospital is a large general hospital serving a population of 800 000. B12 assays were performed upon request without need for justification.

Study Design

All patients with serum B12 measured in 1996 were included. The discharge summaries and case notes of all these patients were reviewed to determine the reasons for B12 measurements. The B12 levels were correlated with the complete blood counts (CBCs) and red cell indices of a blood sample taken within 2 wk before B12 determination. If multiple CBCs were performed, the pretransfusion sample drawn on the date closest to the vitamin B12 measurement was used. Vitamin B12 was measured by microparticle enzyme intrinsic factor assay (Imx System, Abbott Laboratories, Abbott Park, IL, USA). The lower limit of normal is 184 ± 37 pmol/L; therefore, a serum B12 level below 140 pmol/L was defined as definitely deficient and a level between 140 and 184 pmol/L as low with unknown significance. The coefficient of variation for duplicate measurements on the same sample in our laboratory was 5 pmol/L. Pernicious anemia was defined as a low B12 level with a positive Schilling test, positive antiparietal cells, or anti-intrinsic factor antibodies. CBCs were determined with the Cell-Dyne 4000 cell counter (Abbott Laboratories). Anemia was defined as hemoglobin levels below 13 g/dL for men and below 11.5 g/dL for women. The normal MCV range is 81 to 97 fl. Macrocytosis was defined as an MCV above 98 fl, and microcytosis as an MCV below 80 fl. These were based on the reference ranges of the Cell-Dyne cell counter.

Statistical Analysis

Chi-square test was used to compare the percentage of patients with B12 levels below 140, 180, 250, and 300 pmol/L in the different subgroups.

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TABLE I.

	<140 pmol/L	<180 pmol/L	<250 pmol/L	<300 pmol/L
>70 (1817) versus <70 (1924) y	6.6% (120) versus 4.5% (86) 0.001 < P < 0.01	12.9% (235) versus 9.3% (179) P < 0.001	24.2% (440) versus 21.3% (410) 0.03 < P < 0.04	43.4% (789) versus 35.5% (683) P < 0.001
With anemia (2217) versus without anemia (1236)	5.2% (116) versus 6.0% (75) P < 0.2	10.5% (234) versus 11.9% (148) P < 0.2	21.6% (480) versus 24% (298) 0.1 0.1 < P < 0.2	34.6% (768) versus 50% (619) P < 0.001
Macrocytosis (564) versus normocytosis (2268)	9.8% (55) versus 4.6% (104) P < 0.001	13.3% (75) versus 10.4% (237) 0.05 < P < 0.1	25.9% (146) versus 22.7% (515) 0.1 < P < 0.2	40.5% (228) versus 35.6% (807) 0.03 < P < 0.04
Macrocytosis (564) versus microcytosis (621)	9.8% (55) versus 5.2% (32) 0.001 < P < 0.01	13.3% (75) versus 11.3% (70) P < 0.2	25.9% (146) versus 21.2% (131) 0.05 < P < 0.1	40.5% (228) versus 47.7% (296) 0.01 < P < 0.02
Normocytosis (2268) versus microcytosis (621)	4.6% (104) versus 5.2% (32) P < 0.2	10.4% (237) versus 11.3% (70) P < 0.2	22.7% (515) versus 21.2% (131) P < 0.2	35.6% (807) versus 47.7% (296) P < 0.001

* Numbers in parentheses are the number of patients. B12 deficiency was more prevalent in patients older than 70 y (P < 0.01) and with macrocytosis. The interesting observation is that the significant prevalence (≥4.5%) is present in each subgroup.

RESULTS

In 1996, 3741 patients had their serum B12 levels measured, and 3453 patients had corresponding CBC and MCV results. The samples of 288 patients were outside referral levels, and corresponding CBC or clinical records were not available. Two hundred six patients had B12 levels below 140 pmol/L, and 44 patients had pernicious anemia. The results are summarized in Table I (also see Fig. 1).

Age

Of the patients with B12 levels below 140 pmol/L, 6.6% (120 of 1817) were older than 70 y and 4.5% (86 of 1924) were younger than 70. After excluding the 288 patients without CBCs, the results were 6.7% (111 of 1647) and 4.4% (80 of 1806), respectively.

Anemia

Six percent (75 of 1236) of non-anemic and 5.2% (116 of 2217) of anemic patients had B12 levels below 140 pmol/L. Pernicious anemia was more common in the anemic group (36 versus 8). For those older than 70 y without anemia, older than 70 with anemia, younger than 70 without anemia, and younger than 70 with anemia, the results were 7.2% (38 of 526), 6.5% (73 of 1121), 5.2% (37 of 710), and 4% (43 of 1096), respectively.

Macrocytosis Versus Normocytosis Versus Microcytosis

The prevalences of vitamin B12 levels below 140 pmol/L among the three groups were 9.8% (55 of 564), 4.6% (104 of 2268), and 5.2% (32 of 621), respectively. In the anemic subgroups, the results were 8.9% (36 of 321), 3.7% (49 of 1323), and 5.4% (31 of 573). For non-anemic patients, the results were 7.8% (19 of 243), 5.8% (55 of 945), and 2% (1 of 48). Documented pernicious anemia was more common in the macrocytic group (28 versus 16). Of those with B12 levels below 140 pmol/L, 5.6% (9 of 161) had documented thalassemia and MCVs below 70. Of those, four had pernicious anemia. Similarly, 9.8% (5 of 51) of patients with iron deficiency and low MCV had B12 deficiency. Three had confirmed pernicious anemia.

Psychiatric Patients

Only three of the 119 psychiatric patients with normal CBC and red cell indices had B12 levels below 140 pmol/L.

Unclear Indication Group

In 249 patients, the reasons for vitamin B12 measurement were not clear. This group included patients younger than 70 y and those with normal CBCs, normal red cell indices, non-psychiatric presentations, and no risk factor for B12 deficiency. Many of these patients had stroke or ischemic heart disease. Six percent (15 of 249) had B12 levels below 140 pmol/L, with one patient testing positive with the Schilling test.

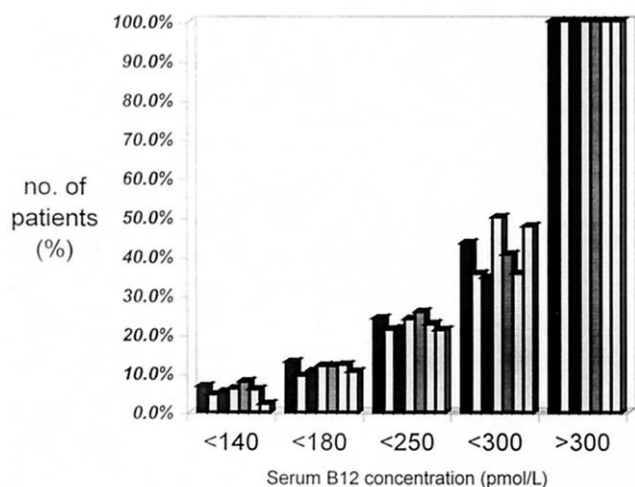


FIG. 1. Summary of the prevalence of vitamin B12 deficiency among different subgroups.

Pernicious Anemia

Only 113 of 382 patients with low B12 levels had Schilling tests, antiparietal cell, or anti-intrinsic factor antibodies performed. Sixty-nine of the 191 patients with B12 levels below 140 pmol/L had further investigations, and 42 had confirmed pernicious anemia with B12 levels between 35 and 102 pmol/L. Among the 191 patients with B12 levels between 140 and 180 pmol/L, 44 had further investigations and two had pernicious anemia with B12 levels of 164 and 171 pmol/L, respectively. Hypersegmented neutrophils were noted in 23 (52%) patients with pernicious anemia.

Hypersegmentation of Neutrophils

Only 87 of the 382 patients with B12 levels below 180 pmol/L had blood film examinations. Hypersegmented neutrophils (six or more lobes) were noted in 23 cases. Eight patients with hypersegmented neutrophils had normal or high B12 levels and were diagnosed as having myelodysplastic syndrome.

DISCUSSION

Traditionally, B12 measurements were recommended for patients with macrocytosis or neurologic or psychiatric symptoms. House officers would be reprimanded for inappropriately ordering B12 levels for patients with hypochromic microcytic anemia and wasting medical resources. Many patients with B12 deficiency have no macrocytosis or hematologic abnormalities.¹⁻⁵ Oosterhuis et al.⁶ analyzed the diagnostic value of an elevated MCV for B12 deficiency. The sensitivity was only 17% to 30%, and up to 84% of the deficiency would be missed. There was no large study of the prevalence of vitamin B12 deficiency among patients with normal or low MCV. We analyzed the clinical records and hematologic parameters of all patients with vitamin B12 measured at our hospital in 1996. There were several interesting observations. The traditional guideline for B12 measurement was not followed in two-thirds of the cases. Among the 3453 patients studied, only 564 had macrocytosis. Another 614 patients had neuropsychiatric problems or risk factors for B12 deficiency. Other studies had reported similar poor adherence to guidelines.^{1,7,8} Many patients with low B12 levels did not have further investigations. Poor follow-up of abnormal laboratory results was not uncommon.^{1,9,10} The true prevalence of pernicious anemia probably would be higher, if appropriate follow-up investigations were performed. Psychiatric patients were frequently screened for vitamin B12 deficiency. Only three of our 119 non-anemic non-macrocytic psychiatric patients had B12 levels below 140 pmol/L. Brett et al.¹¹ observed a similar low prevalence. Among the various subgroups we analyzed— younger versus older than 70 y, anemic versus non-anemic, macrocytosis versus non-macrocytosis—B12 deficiency ranged from 4.8% to 8.9%. Five percent of microcytic patients with thalassemia or iron-deficiency anemia had coexisting B12 deficiency. Hypersegmented neutrophils were reported to be highly sensitive and specific for the diagnosis of megaloblastic anemia in some studies^{12,13} but not in others.¹⁴ Its sensitivity was only 39% in our study. Six percent of patients (15 of 249) without obvious indications for B12 measurements had B12 deficiency, some below 75 pmol/L, with one documented case of pernicious anemia. Many of those B12-deficient patients were younger than 45 y, with histories of stroke or ischemic heart disease. Patients older than 70 y and presenting with severe macrocytosis (MCV > 105) had a high prevalence of B12 deficiency and pernicious anemia. The important observation was not whether vitamin B12 deficiency was more prevalent in any subgroup, but whether a significant prevalence ($\geq 4.5\%$) was observed in each subgroup. Therefore, whatever screening guidelines we used, we would miss a significant number of patients with low B12 values. Because homocysteine levels

were not measured in our patients, those with low B12 values might not be truly deficient. However, Metz et al.¹⁵ showed that almost 90% of elderly patients with serum B12 levels below 150 pmol/L had tissue cobalamin deficiency on the basis of elevated serum homocysteine. Other researchers have suggested that even patients with B12 levels between 150 and 250 pmol/L have subtle deficiency.¹⁶ Fenech¹⁷ associated vitamin B12 deficiency and elevated homocysteine levels with micronucleus formation, and that B12 levels in plasma should exceed 300 pmol/L to minimize DNA damage in blood cells. Patients with methionine synthetase reductase polymorphism (66A \rightarrow G) and those with low transcobalamin II deficiency might have intracellular B12 deficiency and elevated homocysteine levels despite normal B12 levels.^{18,19} If we choose 300 pmol/L as the cutoff point for B12 deficiency, then 35% to 45% of patients would be deficient and might benefit from B12 supplements.

The number of B12 measurements and the percentages of tested patients with anemia, macrocytosis, and normocytosis were fairly consistent each month. On average, 4000 B12 assays were performed annually at our hospital between 1996 and 1999 and about 5% were in the deficient range. Therefore, our observations reflected the hospital population rather than skewed sampling.

B12 deficiency and hyperhomocysteinemia are now considered risk factors for stroke and ischemic heart disease.²⁰⁻²² Correction of hyperhomocysteinemia will have a great impact on health care. Folate supplements are very common nowadays, but folate replacement in B12 deficiency will not prevent neurologic complications. Any screening criteria selected would miss a significant number of B12-deficient patients, so there may be a case for universal B12 screening. How often screening should be repeated in patients with normal values is not known, and patients with "normal levels" may have cellular deficiency. Because oral B12 replacement with high-dose methylcobal is effective even for patients with pernicious anemia and food-bound B12 malabsorption,²³ universal B12 supplements may be a better alternative. A prospective study of vitamin B12 screening and replacement in the general population may clarify this issue.

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