CASES IN PRIMARY CARE LABORATORY MEDICINE

Macrocytosis: pitfalls in testing and summary of guidance

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Accepted: 12 June 2007

BMJ 2007;335:884-6
doi: 10.1136/bmj.39325.689641.471

Low vitamin B-12 levels can occur without deficiency being present, and normal levels don't always rule out vitamin B-12 deficiency. A clinical assessment, together with blood count and blood film results, can ensure a correct interpretation of vitamin B-12 and folate levels

Deficiency of vitamin B-12 and folate classically causes a macrocytic anaemia, but macrocytosis may be due to causes other than deficiency of vitamin B-12 and folate. Neurological changes due to vitamin B-12 deficiency may develop in the absence of changes in the blood count. Incorrect interpretation of vitamin B-12 levels in particular can lead to a wrong diagnosis, inappropriate referral to hospital, and inappropriate investigation. We have published guidance on the indications for requesting vitamin B-12 and folate levels and how to assess response to treatment and follow-up of these patients.¹ The four cases below outline some pitfalls in investigating patients for apparent deficiency of vitamin B-12 and folate and show the value of a pragmatic approach (discussing results with a haematologist and considering a therapeutic trial of vitamin B-12).

Case 1

A 76 year old woman with a diagnosis of polycythaemia rubra vera was under the care of the haematology clinic and was being treated with hydroxycarbamide. She presented to her general practitioner with an intercurrent illness. Her blood count showed haemoglobin 137 g/l, white count $6.7 \times 10^9/l$, platelets $238 \times 10^9/l$, and a mean red cell volume of 127 fl. As a result of the macrocytosis further investigations were done; vitamin B-12 concentration was 457 ng/l (reference range 160-700 ng/l) and red cell folate was 228 µg/l (reference range 97-570 µg/l). In view of the normal results the general practitioner requested an early appointment to investigate the cause of macrocytosis.

Discussion—The level of macrocytosis can predict whether vitamin B-12 and folate deficiency is present.² As the mean cell volume increases to more than 100 fl, the probability of vitamin B-12 and folate deficiency also increases. This is particularly true in patients

with a mean cell volume >130 fl, except for those who are receiving hydroxycarbamide, as illustrated by this case. Patients who are receiving hydroxycarbamide usually have a mean cell volume >110 fl, and the level of the macrocytosis is related to the dose of hydroxycarbamide.³ Mean cell volumes of 100-110 fl are more likely to be related to other causes of macrocytosis, such as alcohol abuse, liver disease, hypothyroidism, anti-neoplastic drugs, HIV infection with the use of zidovudine, and haematological disorders such as haemolysis and myelodysplastic syndromes.^{2 4} A blood film is usually helpful in these cases as hypersegmented neutrophils and macro-ovalocytes are associated with vitamin B-12 and folate deficiency, a uniform macrocytosis with alcohol abuse, target cells with liver disease, and polychromasia with haemolysis.

Case 2

A 32 year old woman who was 24 weeks pregnant presented to her general practitioner with general tiredness. Investigations showed haemoglobin 135 g/l, white cell count $4.8 \times 10^9/l$, platelets $253 \times 10^9/l$, mean cell volume 85 fl, vitamin B-12 140 ng/l (reference range 160-700 ng/l), red cell folate 346 µg/l (normal range 97-570 µg/l), ferritin 23 µg/l (reference range 20-300 µg/l). The patient was referred to the haematology clinic for further assessment in view of the low serum vitamin B-12.

Box 1 Requesting vitamin B-12 and folate concentrations

Vitamin B-12 and folate should be measured in patients with:

- Macrocytic anaemia
- Macrocytosis particularly in patients with a mean cell volume (mean cell volume) >110 fl
- Severe oropharyngeal ulceration or specific neuropsychiatric abnormalities (vitamin B-12 only if no macrocytosis or anaemia) particularly paraesthesiae or proprioceptive loss

Fig 1 | Peripheral blood film in case 3 shows hypogranular neutrophils with abnormal lobulation of the nucleus, indicating that the macrocytic anaemia is due to a myelodysplastic syndrome

Fig 2 | Bone marrow aspirate in case 3 shows blasts, confirming the diagnosis of a myelodysplastic syndrome

Discussion—This case illustrates the importance of attempting to estimate the pretest probability of the target disorder being present before the diagnostic test (vitamin B-12 concentration) is done. This patient had non-specific symptoms with no other relevant history, and vitamin B-12 concentration was requested as part of a general screen. Assessment of vitamin B-12 and folate deficiency should include an assessment of the mean cell volume,² but mean cell volume alone cannot be relied on as a screening test: other pointers in the history should be reviewed, such as family history and neuropsychiatric abnormalities such as paraesthesia, ataxia, peripheral neuropathy, and memory loss. Objective signs associated with vitamin B-12 deficiency include impaired vibration, touch, pain, and position sense together with an abnormal gait.⁵ Other pointers in the history include symptoms of malabsorption or severe oropharyngeal ulceration, which may present without changes in the blood count^{6,7} and respond to treatment with vitamin B-12.⁸ The low vitamin B-12 level in this case was associated with the increased plasma volume that occurs during pregnancy and does not indicate deficiency of vitamin B-12.⁹

Case 3

An 84 year old woman presented in 2005 with a painful knee due to osteoarthritis. At joint replacement she was noted to have a macrocytosis: mean cell volume 102 fl and preoperative haemoglobin 125 g/l. A year later she represented with shortness of breath and tiredness and symptoms of rectal bleeding due to a rectal prolapse, which was repaired. Postoperatively she remained anaemic despite treatment with iron; her haemoglobin level fell gradually to 107 g/l, with a macrocytosis mean cell volume 106 fl. Her serum vitamin B-12 was 250 ng/l (reference range 160-700 ng/l), serum folate 15.5 µg/l

(reference range 3.0-16.0 µg/l), serum ferritin 365 µg/l (reference range 20-300 µg/l). Four months later she was admitted with breathlessness due to left ventricular failure with a blood count that showed haemoglobin 68 g/l and mean cell volume 104 fl. Blood film examination showed hypogranular neutrophils and macrocytes, with no hypersegmented neutrophils (fig 1). A bone marrow aspirate showed features of trilineage dysplasia and increased myeloblasts (7%) consistent with refractory anaemia with excess blasts (RAEB, a category of myelodysplasia) (fig 2). Subsequent treatment included regular monitoring and blood transfusion support via the haematology clinic.

Discussion—This case illustrates the finding of a myelodysplastic syndrome (refractory anaemia) in an elderly patient in whom macrocytosis and hypogranular neutrophils were found before the development of a progressive macrocytic anaemia with normal vitamin B-12 and folate levels. A high proportion of blasts cells (>5%) on marrow examination indicates a high risk of transformation to acute myeloid leukaemia within 12 months. Macrocytic anaemia is relatively common in patients with a myelodysplastic syndrome.

Case 4

An 83 year old woman presented with tiredness and weight loss to her general practitioner. Initial investigations showed haemoglobin 92 g/l and mean cell volume 124 fl, and blood film examination showed hypersegmented neutrophils. Current medication included 30 mg prednisolone daily for polymyalgia rheumatica. She was referred for a gastroenterology opinion: serum B-12 was 360 ng/l (reference range 160-700 ng/l), serum folate 7.4 µg/l (reference range 3.0-16.0 µg/l), and red cell folate 98 µg/l (reference range 95-570 µg/l). In view of the borderline red cell folate results together with the history of weight loss an upper gastrointestinal endoscopy was performed with duodenal biopsy; it showed a hiatus hernia but normal duodenal biopsy with no evidence of coeliac disease. Antigastric parietal cell antibodies were present. A haematologist advised an intrinsic factor antibody assay, and the possibility of a false normal serum vitamin B-12 result was considered. This was confirmed when the intrinsic factor antibody was strongly positive at a very high titre (>100 IU/l), and a pretreatment serum sample was stored for further study. Intramuscular vitamin B-12 resulted in a full haematological response: haemoglobin rose to 132 g/l, mean cell volume fell to 102 fl, and red cell folate corrected to 234 µg/l.

Discussion—Repeat analysis of pretreatment serum vitamin B-12 in this case gave a variety of assay results (table), and two of the five methods found unequivocal low vitamin B-12. The government body that is responsible for ensuring that medical devices are acceptably safe (Medicines and Healthcare Products Regulatory Agency, MHRA), manufacturers, and the UK's National External Quality Assessment Service (UK NEQAS—the body that oversees external quality assurance schemes for pathology laboratories in the UK) scheme for haematologic assays are aware that a false normal vitamin B-12 result may be

Vitamin B12 results (patient 4) measured by five commercial assays

Assay	Reference range (ng/l)	Patient's serum concentration (ng/l)
Abbott architect	162-960	<60
Roche 170	197-866	70
Bayer centaur	160-260 indeterminate <160 deficient	217
Beckman access	145-914	317
DPC immulite 2000	180-800	360

SUMMARY POINTS

Macrocytosis is commonly associated with the use of several drugs and does not require further investigation unless there are additional clinical features

Before testing, the probability of vitamin-B12 or folate deficiency should be undertaken by assessing relevant features in the history and examination

Myelodysplastic syndromes often present as a macrocytic anaemia with normal vitamin-B12 and folate concentrations

When full blood count is normal, vitamin-B12 deficiency should be suspected in patients with neurological signs and severe oropharyngeal ulceration

Box 2 Monitoring patients receiving treatment for vitamin B-12 or folate deficiency

- Initially a full blood count and reticulocyte count after 6-10 days to document the response, and a further check after eight weeks to confirm a normal blood count
- Long term folate monitoring is not necessary unless the cause of the folate deficiency persists
- Vitamin B-12 monitoring is not necessary for patients receiving replacement treatment
- Patients with pernicious anaemia who develop iron deficiency should be referred for endoscopy to exclude gastric carcinoma

caused by interference by high titre intrinsic factor antibody. In a similar case described recently, the patient presented with a marked macrocytic anaemia and normal serum vitamin B-12 levels and responded promptly to treatment with vitamin B-12.¹⁰ Clinicians also need to be alert to the possibility of megaloblastic anaemia or subacute combined degeneration of the cord with normal B-12 results, which may be suspected by the presence of high titre intrinsic factor antibody.

Megaloblastic anaemia or subacute combined degeneration of the cord can occur with normal B-12 results, and may be suspected when high titre intrinsic factor antibody is present. Any further cases should be reported to the MHRA and UK NEQAS scheme for haematinic assays, and a pretreatment serum sample should be stored. Where vitamin B-12 deficiency is suspected, intramuscular vitamin B-12 should be tried, and clinical and haematological response should be evaluated 6-10 days after treatment by a repeat blood count and reticulocyte count. The UK NEQAS scheme for haematinic assays is aware of nine similar cases of false normal vitamin B-12 levels in either megaloblastic anaemia or subacute combined degeneration of the cord. Manufacturers of vitamin B-12 assays warn that the diagnosis of deficiency should not rely on a single cobalamin assay result; it requires evaluation of other supportive laboratory tests, together with a clinical evaluation.

Other laboratory tests have been suggested for identifying functional vitamin B-12 deficiency in patients who have clinical symptoms or signs of deficiency with normal levels of vitamin B-12. However two recent studies have shown that tests for methylmalonic acid, homocysteine, and holotranscobalamin have a limited role in routine laboratory practice.^{11 12} In patients like the one in case 4, macrocytic anaemia

and a blood film showing hypersegmented neutrophils were sufficient for starting treatment with vitamin B-12. In patients who present with macrocytic anaemia and low vitamin B-12, these additional tests are not superior to vitamin B-12 levels in predicting a haematological response to treatment with vitamin B-12.¹¹ Samples for homocysteine levels, for example, should be collected in carefully controlled situations; also, these additional metabolic tests are performed only in specialist laboratories in the United Kingdom.

Evidence note

This guidance is drawn from consensus guidelines and observational studies. The consensus guidelines emphasise the importance of attempting to assess whether a deficiency is present before requesting vitamin B-12 and folate levels.² The important features to assess are medication, family history, diet, alcohol intake, symptoms of malabsorption, and other conditions such as liver disease or hypothyroidism. An examination of a blood film will be useful in difficult cases. Observational studies have assessed whether additional tests for methylmalonic acid, homocysteine, and holotranscobalamin can contribute to the interpretation of vitamin B-12 levels, but no clear consensus has emerged.^{5 11 12} These tests need special collection and processing of the sample for the homocysteine levels, and they are performed in specialist laboratories.

We have published guidance on the indications for requesting vitamin B-12 and folate levels and how to assess response to treatment and follow-up of these patients¹; this is summarised in the boxes.

We thank D A Bareford, who co-authored the original guidance.

Contributors: Both authors contributed equally to the writing of this paper.

Competing interests: None declared.

Provenance and peer review: Not commissioned; externally peer reviewed.

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Useful websites

Lab tests online (www.labtestsonline.org.uk)—A comprehensive guide to laboratory tests and their use for patients

Cochrane Library (www.nelh.nhs.uk/cochrane.asp)—Information and systemic reviews on evidence based medicine. The Cochrane Collaboration is beginning reviews on laboratory diagnostic testing

Journal of Clinical Pathology (www.jclinpath.com)—Subscription website containing electronic access to the Journal of Clinical Pathology with full content of the questions and answers examined in this article

Clinical Evidence (www.clinicalevidence.com)—Summaries of current evidence based management guidelines

PRODIGY (www.prodigy.nhs.uk)—Clinical decision making guidelines principally for general practitioners