Diagnosing vitamin B-12 deficiency on the basis of serum B-12 assay

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Lesson of the week
Diagnosing vitamin B-12 deficiency on the basis of serum B-12 assay
Vinod Devalia

Serum vitamin B-12 concentrations are measured to assess the presence of its deficiency in patients presenting with haematological, neurological, and neuropsychiatric abnormalities. Replacement therapy is instituted promptly, particularly to prevent irreversible neurological and cognitive dysfunction.

I present two cases with paradoxical vitamin B-12 results, which highlight the fallacies of the serum vitamin B-12 assay and emphasise the importance of taking into account the overall clinical picture before prejudging the significance of the vitamin B-12 assay result.

Case reports
Case 1
A 59 year old white woman was seen urgently for assessment of a macrocytic anaemia. She had normal serum B-12 concentrations, confirmed on three occasions. She complained of progressively increasing lethargy, palpitations, and buzzing in the ears over about three months. She had a good, well balanced diet and was not a vegetarian. Apart from thyroxine, she was taking no regular medication. She said her father had had pernicious anaemia. On clinical examination the only clinically significant findings were a mild glossitis and pallor. A full blood count showed a substantial macrocytic anaemia and a mild reduction of the white cell count (figure). The blood film showed mild oval macrocytosis, occasional nucleated red cells, and some hypersegmented neutrophils. An urgent bone marrow examination showed megaloblastic haemopoiesis.

The most likely diagnosis, in the face of normal serum vitamin B-12 concentration, would have been myelodysplastic syndrome needing urgent blood transfusion and further haematology assessment. However, in view of her history of thyroid disease and family history of pernicious anaemia, an underlying functional vitamin B-12 deficiency could not be entirely discounted. A blood sample for autoantibody screen, anti-intrinsic factor antibody, and serum homocysteine and methylmalonic acid were taken and she was started immediately on replacement vitamin B-12 therapy pending these results.

She had a prompt response, with her full blood count returning to normal within eight weeks of therapy (haemoglobin 120 g/l; mean cell volume 87.5 fl) without the need for blood transfusion. The laboratory results and bone marrow appearances for the cases presented. Case 1 shows a normal serum vitamin B-12 concentration with biochemical features of B-12 deficiency (raised homocysteine and methylmalonic acid). The haematology indices (macrocytic anaemia) and bone marrow appearances are consistent with megaloblastic anaemia caused by classic pernicious anaemia, confirmed by a positive anti-intrinsic factor antibody. Case 2 shows a markedly reduced serum vitamin B-12 concentration but largely unremarkable biochemical features (normal homocysteine concentration and slightly raised methylmalonic acid) of vitamin B-12 deficiency. The mild anaemia and reduced mean cell volume are consistent with a β thalassaemia trait, and the bone marrow appearances are normal.

Case 2
A 60 year old white woman with β thalassaemia trait had her serum vitamin B-12 concentration checked as she mentioned that she was a vegan. She had no symptoms of note and gave a history of avoiding meat and milk products for the past eight years. She had no family history of pernicious anaemia or other autoimmune conditions. She was not taking any medications. Her serum vitamin B-12 concentration was markedly reduced (figure)—confirmed on three occasions—with a haemoglobin concentration at the lower end of the

<table>
<thead>
<tr>
<th>Test</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folate (6.7-45.3) (nmol/l)</td>
<td>13.1</td>
<td>17.7</td>
</tr>
<tr>
<td>Haemoglobin (115-165) (g/l)</td>
<td>76</td>
<td>115</td>
</tr>
<tr>
<td>Mean cell volume (78.8-99.0) (fl)</td>
<td>126.5</td>
<td>71.5</td>
</tr>
<tr>
<td>White cell count (4.0-11.0) (x10^9/l)</td>
<td>3.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Platelets (150-450) (x10^9/l)</td>
<td>156</td>
<td>167</td>
</tr>
<tr>
<td>Plasma homocysteine (0.0-16.0) (µmol/l)</td>
<td>44.9</td>
<td>12.5</td>
</tr>
<tr>
<td>Plasma methylmalonic acid (0.10-0.42) (µmol/l)</td>
<td>2.10</td>
<td>0.87</td>
</tr>
<tr>
<td>Autoantibody screen</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-intrinsic factor antibody</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Laboratory results and bone marrow appearances for the cases presented. Case 1 shows a normal serum vitamin B-12 concentration with biochemical features of B-12 deficiency (raised homocysteine and methylmalonic acid). The haematology indices (macrocytic anaemia) and bone marrow appearances are consistent with megaloblastic anaemia caused by classic pernicious anaemia, confirmed by a positive anti-intrinsic factor antibody. Case 2 shows a markedly reduced serum vitamin B-12 concentration but largely unremarkable biochemical features (normal homocysteine concentration and slightly raised methylmalonic acid) of vitamin B-12 deficiency. The mild anaemia and reduced mean cell volume are consistent with a β thalassaemia trait, and the bone marrow appearances are normal.

The clinical picture is of utmost importance when interpreting a vitamin B-12 assay result.
normal range, consistent with her thalassaemia trait. On examination, there were no clinical features of note. Her anti-intrinsic factor antibody was negative and her serum ferritin concentration was within normal limits. Conventionally, she would have been given a course of vitamin B-12 injections immediately, and subsequently a Schilling test (or equivalent vitamin B-12 absorption study) would have been performed. In view of her general wellbeing, a bone marrow examination was done to evaluate any effect on erythropoiesis as a marker for functional vitamin B-12 deficiency. The bone marrow showed normal erythropoiesis, her plasma homocysteine was normal, and her methylmalonic acid was raised sufficiently to indicate possible subclinical deficiency.7

Discussion

The diagnosis of vitamin B-12 deficiency has always been problematic owing to the unavailability of a robust assay.8 Normal concentrations of vitamin B-12 have been reported in patients with overt deficiency,9 and the type of assay used may be relevant.10 We use the Access Immunoassay System (Beckman Coulter) which has shown a coefficient of variation of 6-10% under the United Kingdom National External Quality Assessment Scheme in the recent past. This implies that the results obtained in the cases presented are qualitatively and quantitatively in defined categories of “normal” in case 1 and “deficient” in case 2.

Case 1 highlights the dangers of a “false normal” vitamin B-12 result. The existence of a large “grey area” for vitamin B-12 assays with regard to interpreting deficiency is well recognised. However, the repeated (three) results showing vitamin B-12 values in the upper part of the normal range in this patient is unusual. There may be unique analytical reasons in this patient (for example, heterophil antibodies), although this remains speculative. Despite a normal result, a diagnosis of vitamin B-12 deficiency was considered possible, and so treatment started straightaway. The more “logical” diagnosis—of megaloblastic anaemia—would have led to her having a much more aggressive and potentially detrimental therapy combining chemotherapy and bone marrow transplantation.

In case 2, the vitamin B-12 concentration was particularly low despite the absence of clinical symptoms and normal bone marrow appearances. Possibly the patient is developing vitamin B-12 deficiency.11 Last year about 8500 serum vitamin B-12 assays from primary and secondary care were done in our haematology laboratory, which serves a population of 290 000. The number of assays is increasing annually by an average of 9%. Testing may be done as part of an annual examination to evaluate any effect on erythropoiesis as a marker for functional vitamin B-12 deficiency in a screening situation12 and to demonstrate biochemical benefit in such patients given vitamin B-12 treatment empirically.13 However, a recent study of patients in an ambulatory setting also raised the question of whether homocystine and methylmalonic acid were as reliable as generally believed.14 Measurement of holotranscobalamin II, another recent marker for functional vitamin B-12 concentrations, is claimed to be more sensitive than methylmalonic acid15 but its clinical utility is open to question.16

Ultimately, meticulous clinical assessment—including assessment of other autoimmune conditions and taking a family history—is important, given that a single ideal test is still not available. Testing for anti-intrinsic factor antibody, despite a normal serum vitamin B-12 concentration, can be particularly useful when underlying pernicious anaemia is strongly suspected, as in case 1. However, a reduced serum vitamin B-12 concentration associated with a negative anti-intrinsic factor antibody, as in case 2, may not help in arriving at a diagnosis as only half of patients with pernicious anaemia are positive for this. Empirical treatment, to assess any clinical response and to prevent neurological damage, may be pragmatically justifiable because the dangers of treatment are not as devastating as those of not treating.

I thank the staff at the University Hospital of Wales, Cardiff, who carried out methylmalonic acid and homocysteine assays in the two patients described, and Gareth Davies for taking photographs of the bone marrow.

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