

CASE REPORT

Dietary folate deficiency with normal red cell folate and circulating blasts

G L Stark, P J Hamilton

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This report describes a 26 year old woman, of Pakistani origin, who presented five months postpartum with severe megaloblastic anaemia as a result of nutritional folate deficiency. This case was unusual in that a small number of myeloblasts were present in the peripheral blood at presentation, and this circulating population temporarily increased in size when folate replacement was begun. We also highlight the need to recognise the non-linear relation between haematocrit and red blood cell folate concentration when the haematocrit is very low (<0.15) and emphasise the importance of the clinical history.

The 20th century saw important and fundamental advances in our understanding of the pathogenesis and treatment of the megaloblastic anaemias. In 1926, Minot and Murphy¹ discovered that pernicious anaemia, a previously fatal disease, could be treated by the ingestion of liver. Vitamin B12, which is the vitamin responsible, was subsequently isolated in 1948.^{2,3} Folic acid, originally called Wills's factor, was discovered in 1931 when Dr Lucy Wills,⁴ studying nutritional anaemias in India, observed that the macrocytic anaemia of pregnancy was more common in poorer women and was corrected by dietary supplements of yeast or yeast extract (Marmite). Seventy years later, megaloblastic anaemia still holds surprises.

CASE REPORT

Presenting features

A 26 year old woman, originally from Pakistan and living in the UK for two years, presented feeling extremely weak and tired. At the time of presentation she was five months postpartum and continuing to breast feed her healthy son. She was a vegetarian but ate very little. Her main food source was chapattis, which she ate with no accompaniments, and very small amounts of fruit. The patient had been taking ferrous sulfate (200 mg daily) for 10 months because normochromic normocytic anaemia had been noted during pregnancy. No haematinic assays were performed at this time. There was no other relevant past medical history. Physical examination revealed no abnormalities except a tachycardia and extreme pallor.

Investigations included a full blood count, which revealed: haemoglobin (Hb), 28 g/litre; mean cell volume (MCV), 100 fl; no reticulocytes present; white blood cell count (WBC), 1.4×10^9 /litre (neutrophils, 0.3×10^9 /litre), and platelets 16×10^9 /litre. Blood film examination showed gross anisopoikilocytosis, tear drop poikilocytes, and basophilic stippling of erythrocytes. A small population of myeloblasts was identified in the peripheral blood (fig 1A–C). In addition, circulating megaloblasts were easily found (fig 1D), although there was no neutrophil hypersegmentation. The bone marrow aspirate was hypercellular. Erythropoiesis was greatly expanded, left

shifted, and megaloblastic. Granulopoiesis was also hyperplastic and giant metamyelocytes were present. There was no excess of myeloblasts. The bone marrow trephine specimen was hypercellular at 95% with loss of fat spaces. The hyperplastic, left shifted erythroid series was again prominent. Occasional giant metamyelocytes were seen and megakaryocytes were normally represented.

Red blood cell folate (RCF) was measured in the normal range at 222 µg/litre (normal range, 160–600). After correction for serum folate the RCF was 200 µg/litre. Serum folate was low at 1.9 µg/litre (normal range, 3.3–13), as was vitamin B12 at 70 ng/litre (normal range, 170–700), although the ferritin concentration was normal. The lactate dehydrogenase (LDH) concentration was very high at 6410 U/litre, with a slightly raised bilirubin at 29 µmol/litre, and reduced haptoglobins. Other liver function tests were normal. Anti-intrinsic factor antibodies and anti-endomysial antibodies were not detected. A Schilling test, undertaken three months after presentation, was normal.

Response to treatment

Given the severity of the pancytopenia, we began vitamin B12 and folate replacement before the haematinic assay results were known, discontinuing the vitamin B12 after three injections, and continuing replacement with folic acid alone for four months. The reticulocyte response was dramatic, reaching a maximum of 608×10^9 /litre on the fourth day after treatment. Hypokalaemia has previously been recognised as an important problem in the treatment of megaloblastic anaemia and is an important cause of death in such patients.⁵ In our patient, the potassium concentration dipped below normal on day 2 of treatment; potassium supplements were begun and continued for one week. Six weeks after starting treatment, the patient had a normal blood count with: Hb, 124 g/litre; MCV, 87 fl; WBC, 8.9×10^9 /litre; and platelets, 371×10^9 /litre.

DISCUSSION

The morphological features seen in this case are, in the main, consistent with those of megaloblastic anaemia resulting from vitamin B12 or folate deficiency. These features have been well described previously.⁶ However, we were concerned by the presence of circulating myeloblasts in our patient and the bone marrow aspirate was crucial in demonstrating no excess of blasts in the bone marrow. It is almost 50 years since six similar cases of folate deficiency in the puerperium were described, in which myeloblasts were present in the peripheral blood at diagnosis.^{7–9} In three of these patients,^{8,9} and in our patient, the leukaemoid reaction became more pronounced in the first few days after starting folate replacement.

Abbreviations: Hb, haemoglobin; LDH, lactate dehydrogenase; MCV, mean cell volume; RCF, red blood cell folate; WBC, white blood cell count

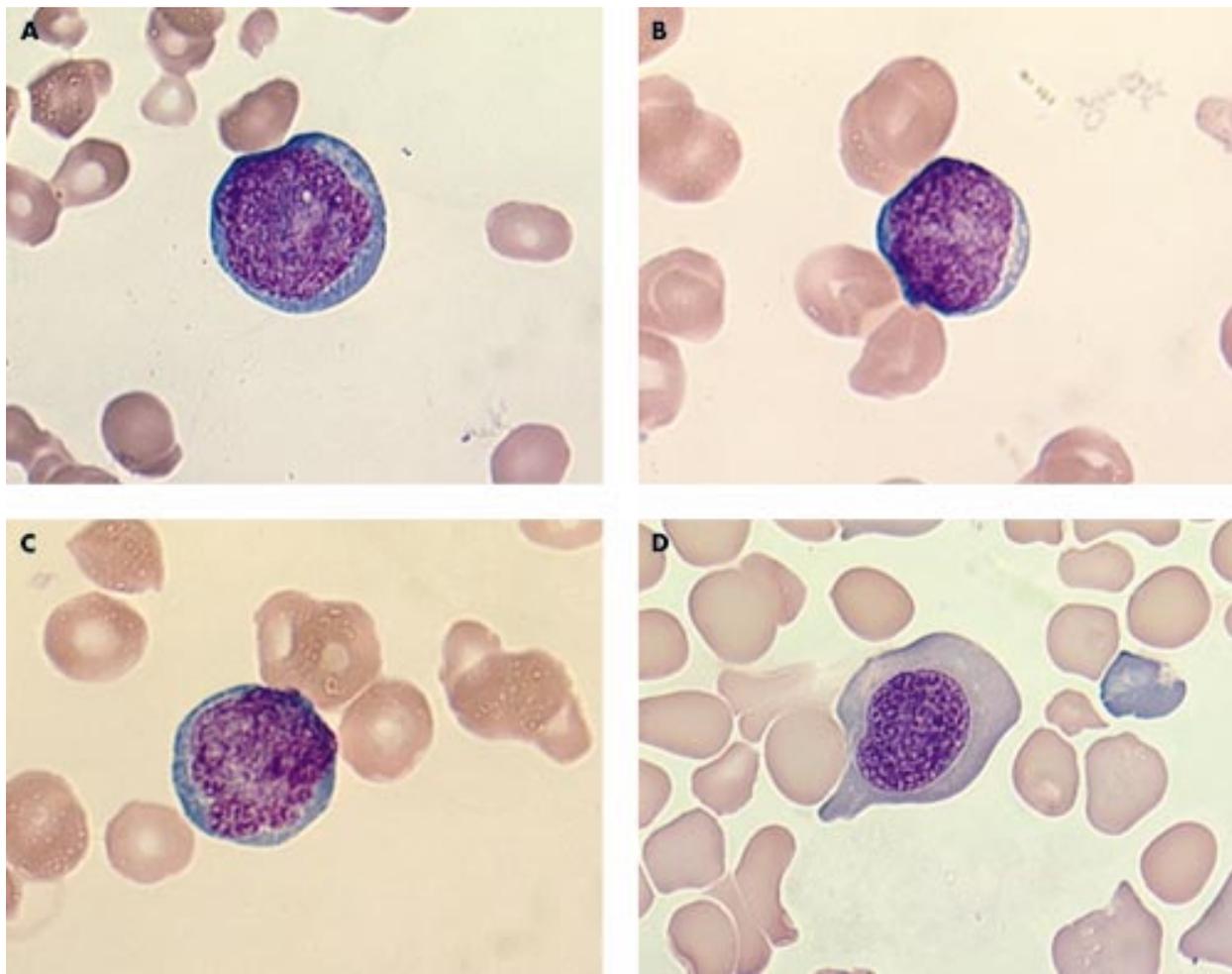


Figure 1 Cells in the peripheral blood (Romanowsky stain). (A–C) Cells with the morphological appearance of myeloblasts and (D) a megaloblast.

In view of the recent pregnancy, ongoing lactation, poor diet, and precipitate presentation, we felt that the probable cause of the megaloblastic anaemia was folate deficiency. Although dietary vitamin B12 deficiency may have played a part, subsequent follow up appeared to support a diagnosis of folate deficiency because the patient continued to follow a strict vegetarian diet and has had no recurrence of vitamin B12 deficiency in the absence of vitamin B12 replacement. Raised LDH and bilirubin, along with reduced haptoglobin values, can be explained by ineffective erythropoiesis and excess intramedullary cell death. These features are well recognised in both severe vitamin B12 and folate deficiency.⁶ The low serum folate and serum vitamin B12 values are consistent with folate deficiency. Mollin and colleagues¹⁰ found serum vitamin B12 concentrations of < 100 ng/litre in 15 of 142 patients with folate deficiency. In view of the severe megaloblastosis, we were surprised by the normal RCF results, but believe that these can be explained by limitations of the folate assay system.

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To measure red blood cell and serum folate we use an ion capture assay with fluorimetric quantitation (Axsym; Abbott Laboratories, Abbot Park, Illinois, USA). Serum folate is assayed along with the total folate in a haemolysate of whole

blood. Therefore, the total blood folate so measured equals RCF plus serum folate. The mean red blood cell folate concentration in the cells (RCF) is proportional to total blood folate divided by the haematocrit. The contribution from serum folate is assumed to be insignificant compared with the red blood cell folate contribution.

We believe that the normal red blood cell folate result is spurious and can be explained by limitations in the technique. First, any error or variability in the folate assay will be magnified by the extremely low haematocrit (0.08 in this case). Second, the contribution of serum folate to the total blood folate needs to be considered. Serum folate is assumed to have little impact on red blood cell folate measurements because the folate concentration in red cells is 20–50 times that in serum. In this case, however, the ratio of red blood cells to serum is greatly reduced, so the proportion of serum folate to total folate will be higher, despite the serum folate measurement itself being low. Notably, the red blood cell folate concentration remained in the normal range, in this case, even after correction for serum folate values. Bain and colleagues¹¹ found a non-linear relation between RCF values and haematocrit, with RCF measurements significantly higher than expected, particularly when the haematocrit was < 0.15, and that this effect persisted even after correction for serum folate values.

CONCLUSIONS

We present a case of severe megaloblastic anaemia resulting from nutritional folate deficiency. We report the unusual feature

Take home messages

- We describe a 26 year old woman who presented five months postpartum with severe megaloblastic anaemia as a result of nutritional folate deficiency
- The case was unusual in that a small number of myeloblasts were present in the peripheral blood at presentation, and this circulating population temporarily increased in size when folate replacement was begun
- This case highlights a pitfall in red cell folate measurement, namely: the non-linear relation between haematocrit and red blood cell folate concentration when the haematocrit is very low (< 0.15)
- We also stress the importance of the clinical history rather than over reliance on laboratory tests and the need for close cooperation between clinical teams and laboratory staff

of circulating blasts in the peripheral blood at presentation and re-emphasise the importance of recognising a pitfall in red cell folate measurement.

Our case highlights the importance of the clinical history rather than over reliance on laboratory tests. It also emphasises the ongoing need for close cooperation between clinical teams and laboratory staff. There is no doubt that an understanding of laboratory methods and their limitations is necessary for the useful interpretation of the many investigations that we request daily.

Although our understanding of the anaemias of pregnancy and the puerperium has undoubtedly increased since the descriptions of Sir William Osler in 1919,¹² it is clear that life threatening, postpartum nutritional anaemias can occur in a "First World" country even in the 21st century. It is vital that in these days of ever quickening medical progress, we do not lose sight of the fundamental lessons of the last century.

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Authors' affiliations

G L Stark, P J Hamilton, Department of Haematology, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP, UK

Correspondence to: Dr G L Stark, Department of Haematology, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP, UK; g.l.stark@ncl.ac.uk

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