Diagnostic and management strategies for anaemia in adults

BACKGROUND Anaemia is often an incidental finding or is discovered when patients present with nonspecific symptoms such as tiredness.
OBJECTIVE This article presents strategies to identify the cause of anaemia and uses four case studies to illustrate these principles.
DISCUSSION Few diagnostic tests can completely rule in or rule out causes of anaemia. This makes the investigation of anaemia complex. An understanding of the limitations of tests in aiding diagnosis is required.

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Anaemia is often detected incidentally or as a result of vague symptoms such as tiredness. Often there are few clues in the history or the examination to the cause of the anaemia and the general practitioner has to rely on the results of further blood tests. Knowing which further tests to order and how to interpret the results can be difficult. The cases below illustrate some of these complexities, in particular the ability of tests to rule in or rule out potential causes of anaemia.

The reference values for haemoglobin concentration are based on population data (*Table 1*), ie. some patients will have low haemoglobin values with no underlying disease. The probability of establishing a clinically important cause is more than 50%.[1] Although this means that no cause is found in a considerable proportion of patients, all detected cases should be investigated in order to exclude potentially serious and treatable disorders. Screening for anaemia is not recommended as patients with mild anaemia who are asymptomatic do not benefit from treatment[2] and the yield of clinically significant pathology is low.[3]

Case 1 – Sarah M

Sarah M is a 38 year old woman who presents with fatigue. She eats a primarily vegetarian diet and has done so for the past 10 years. The results of her full blood count (FBE) are Hb 84 g/L and mean cell volume (MCV) 110 fL. There are some hypersegmented nuclei in the neutrophils on the peripheral blood film.

What is the most likely cause of Sarah's anaemia?

The most common cause of macrocytosis is alcohol. Even small quantities of alcohol can raise the mean cell volume, even if haemoglobin levels remain normal.[4] The most common cause of megaloblastic anaemia is pernicious anaemia. Rates vary between locations, but in the United Kingdom about 80% of megaloblastic anaemia is due to pernicious anaemia.[4] The incidence peaks at 60 years of age and is associated with other autoimmune

Table 1. Adult reference values for haemotological indices[21]

Haemoglobin:	male	130–180 g/L
	female	115–165 g/L
Mean cell volume		80–100 fL
Vitamin B12		120–680 pmol/L
Red cell folate		360–1400 nmol/L
Serum folate		7–45 nmol/L
Serum ferritin:	male	30–300 μg/L
	female	15–200 μg/L

diseases (vitiligo, Hashimoto disease, Addison disease and hypoparathyroidism). Dietary deficiency of vitamin B12 is an uncommon cause unless eating a strictly vegan diet. Malabsorption of B12 due to atrophic gastritis may be a cause of B12 deficiency in the elderly or in patients who have had gastric surgery. Folate deficiency is most commonly associated with alcoholism and poor nutrition or antifolate medications. Other causes of macrocytosis are shown in *Table 2*.

What other examinations should be performed?

Patients with B12 deficiency are at risk of a peripheral neuropathy (subacute combined degeneration). This affects the posterior and lateral columns of the spinal cord and the peripheral nerves, affecting the legs more than the arms. The neuropathy can occur in the absence of anaemia. The patient may have symptoms of numbness and tingling. The neurological signs are disturbance of position and vibration sense and up-going toes.

Vitamin B12 deficiency can also cause memory loss, depression and dementia. This can occur with no peripheral nerve involvement or anaemia. Other symptoms are anorexia and diarrhoea. Atrophic glossitis is often listed as a classic sign, but also occurs in iron deficiency and other nutritional deficiency states.[5] Neurological deficits do not occur in folate deficiency.

What tests should be ordered?

The initial investigations for megaloblastic anaemia should be serum B12 assay, serum and red cell folate assay and, if B12 deficient, parietal cell and intrinsic factor antibodies. The probability of B12 and folate deficiency is in proportion to the degree of macrocytosis. The greater the elevation in mean corpuscular volume, the greater the probability that the cause of the anaemia is due to either B12 or folate deficiency. In patients with levels greater than 130 fL, the cause is nearly always B12 or folate deficiency.[6] Conversely, the absence of macrocytosis does not exclude B12 deficiency.[7]

There are many possible causes for false positive and false negative B12 and folate level results. Vitamin B12 levels can be affected by recent antibiotic usage or vitamin intake, and folate deficiency can also cause an apparently low B12 level.[8] Patients with pernicious anaemia can have serum B12 levels within the normal range (ie. >120 pmol/L), so should be retested if the diagnosis remains uncertain. The most accurate measure of body folate levels is red blood cell folate levels. A recent intake of green vegetables can increase the serum folate level. Vitamin B12 deficiency can also cause a falsely low red blood cell folate level, often with a coexisting elevated serum folate level.

Intrinsic factor antibodies are present in 50% of patients with pernicious anaemia, but have high specificity so if present confirm the diagnosis. Parietal cell antibodies occur in 90% of patients with pernicious anaemia, but also in up to 22% of healthy persons.[9] If the cause is uncertain, serum methylmalonic acid and total homocysteine levels may assist in the diagnosis. Elevation of both metabolites is a highly sensitive indicator for B12 deficiency, whereas only homocysteine is elevated in folate deficiency.[10] The Schilling test had poor sensitivity and is no longer generally available in Australia. Sarah had a serum B12 level of 98 pmol/L and intrinsic factor antibodies were present.

What is the management for Sarah?

The traditional management of B12 deficiency is parenteral replacement commencing with six injections of hydroxocobalamin 1 mg every 3–4 days, and then at three monthly intervals for life. Oral replacement is as effective, using a dosage of 1000 μg per day.[11,12] It is often quoted that folate should be avoided in patients with B12 deficiency as it may precipitate worsening of the neurological symptoms, but this appears to be based on case reports.[13] An endoscopy should be performed to exclude gastric and carcinoid tumours as these are 2–3 times more common in patients with perni-

cious anaemia than in age and sex matched controls.[4] A study following 56 patients for three years detected two gastric cancers and two carcinoid tumours, a number needed to test of 14.[14]

Table 2. Differential diagnosis of macrocytosis

Alcohol	
Megaloblastic anaemia	Vitamin B12 deficiency – diet, pernicious anaemia, gastrectomy
	Folate deficiency – diet, coeliac disease, anticonvulsants
Drugs	Chemotherapy, azathioprine, acyclovir, methotrexate, anticonvulsants, metformin, colchicine
Liver disease	
Haemolytic anaemia (resulting in reticulocytosis)	
Myelodysplasia	
Myeloma	
Hypothyroidism	
Aplastic anaemia	
Red cell aplasia	

Case 2 – David M

David M is a 36 year old man who presents with fatigue and malaise. He has experienced ongoing symptoms for several years and cannot say when he last felt well. He eats a vegetarian diet, exercises regularly and works as a gardener. The results of his FBC are Hb 86 g/L and MCV 105 fL. There were a small number of hypersegmented neutrophils in the blood smear.

What is the most likely cause of David's anaemia?

Pernicious anaemia is again a possibility, but the patient's age and gender make this diagnosis less likely. The tests described earlier were performed and the patient was found to have a red cell folate level of 280 nmol/L and a serum folate level of 5 nmol/L. Serum B12 levels were normal and David was commenced on folate replacement. A follow up blood test was performed after two months and the results showed Hb 95 g/L and MCV 98 fL. The peripheral blood smear showed microcytes, macrocytes, hypersegmented neutrophils and Howell-Jolly bodies. The diagnostic significance of features seen in peripheral blood smears is shown in *Table 3*.

Table 3. Diagnostic significance of features seen in peripheral blood smears

Anisocytosis (variation in size)	Megaloblastic anaemia, iron deficiency anaemia, thalassaemia
Basophilic stippling of red blood cells	Thalassaemia traits, lead poisoning, dyserythropoiesis
Blast cells	Myelofibrosis, leukaemia
Heinz bodies	G6PD deficiency
Howell-Jolly bodies (nuclear remnants)	Splenectomy, rarely in leukaemia, megaloblastic anaemia, iron deficiency anaemia
Hypersegmented neutrophil nuclei	Megaloblastic anaemia, uraemia, liver disease
Oval macrocytes	A wide variety of anaemic conditions, eg. megaloblastic anaemia, iron deficiency anaemia, thalassaemia. Red cells are oval in about 1% of nonanaemic people
Poikilocytosis (variation in shape)	Megaloblastic anaemia, iron deficiency anaemia, lead poisoning, acute haemolytic anaemia
Polychromasia (uneven staining)	Younger red blood cells stain blue so causes are bleeding, haemolysis, iron and B12 replacement, dyserythropoiesis
Schistocytes (fragment of red blood cells)	Intravascular haemolysis
Sideroblasts (normoblasts with stainable iron)	Haemolytic anaemia, megaloblastic anaemia, haemochromatosis, haemosiderosis
Spherocytes	Auto-immune haemolysis, hereditary spherocytosis
Target cells	Liver disease, thalassaemia, sickle cell disease and occasionally in iron deficiency anaemia

What tests should be performed now?

David should have a more complete work-up for anaemia, including iron studies and testing for haemolytic anaemia. In cases of refractory anaemia, particularly in the presence of iron and/or folate deficiency anaemia, the diagnosis of coeliac disease should be considered, as anaemia is common as the presenting feature of the disease. Coeliac disease is a relatively common disorder, occurring in about 1% of participants in a prevalence study conducted in the United Kingdom,[15] but it is five times more common in patients with iron deficiency anaemia. Another prospective study identified coeliac disease in 5% of patients diagnosed with iron or folate deficiency anaemia.[16] Therefore, it is important to consider this diagnosis in patients with otherwise unexplained or refractory iron or folate deficiency anaemia. The absence of gastrointestinal symptoms such as diarrhoea and weight loss does not exclude the diagnosis.[17]

Serum tests (IgA and IgG antigliadin antibodies and anti-endomysial antibodies) can be used to rule out coeliac disease as they have high sensitivity.

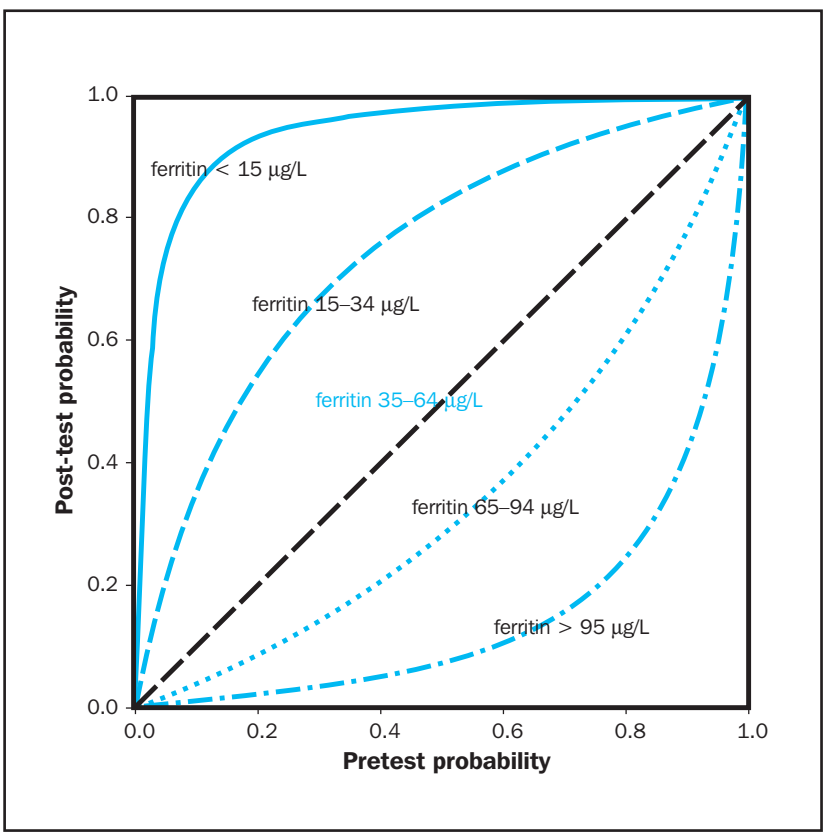

Figure 1. Post-test probability of iron deficiency anaemia after ferritin testing[19]

Approximately 10% of patients with iron and/or folate deficiency test positive to at least one of these antibody tests, of whom 50% are confirmed to have coeliac disease on small bowel biopsy.[16]

What is the management of David?

Endoscopy and small bowel biopsy confirms coeliac disease. David should commence a gluten free diet. Haemoglobin and mean corpuscular volume can be monitored for response to therapy.

Case 3 – Maria D

Maria D is a 65 year old woman who presents with fatigue and shortness of breath. She has a history of heart failure with mild renal failure. Her FBC results are Hb 98 g/L and MCV 80 fL.

What is the most likely cause of Maria's anaemia?

There are two likely causes in this case: iron deficiency anaemia due to chronic blood loss or anaemia of chronic disease. The main causes of anaemia of chronic disease are infection, inflammation, neoplasia and chronic renal disease.[18] The anaemia of chronic disease can cause hypochromia and microcytosis, ie. similar blood test results to iron deficiency. Frequently the two conditions will coexist. In developing countries, the most common cause of iron deficiency is hookworm infestation. Other causes of microcytic anaemia are thalassaemia and sideroblastic anaemia.

What investigations should be performed?

The first step in this case should be to review the history and clinical findings of the patient to identify any potential sources of chronic blood loss. In this age group, the most common cause is a gastrointestinal malignancy, but chronic blood loss may also be due to medications such as aspirin or nonsteroidal anti-inflammatory medication.

The most sensitive and specific indicator of body iron stores is serum ferritin and only this part of the iron studies needs to be ordered. If serum ferritin is low, it rules in the diagnosis of iron deficiency anaemia. If serum ferritin is normal or raised, the most likely cause of the anaemia is chronic disease. Ferritin results can be confusing, however, as the reference range for normal values is wide (*Table 1*). *Figure 1* shows the ability of different levels of

serum ferritin to rule in or rule out the diagnosis.[19] Given the relatively high pre-test probability of iron deficiency anaemia in this case,[19] even if the ferritin result is as high as 90 g/L, the probability that Maria has iron deficiency anaemia is still 14% (see *Example*).

Example

The probability a patient has a disease can be calculated using the formula:

Post-test odds = likelihood ratio x pretest odds

$$\text{Post-test probability} = \frac{\text{likelihood ratio x pretest odds}}{\text{likelihood ratio x pretest odds} + 1}$$

A 65 year old woman with microcytic anaemia has a pretest probability of iron deficiency anaemia of 0.3 (30%) = pre-test odds of 0.43. A serum ferritin value of 90 μg/L has a positive likelihood ratio of 0.39.

The probability of iron deficiency anaemia given a serum ferritin level of 90 μg/L:

$$= \frac{(0.39 \times 0.43)}{(0.39 \times [0.43 + 1])}$$

= 14%

Both iron and ferritin show changes in acute phase reactions, with iron falling and ferritin being elevated. This can cause difficulties with interpretation of the iron studies in the presence of infection, liver disease or inflammation. If there is still diagnostic confusion, a therapeutic trial of oral iron therapy should be considered, monitoring the reticulocyte response at 7–10 days.

Case 4 – Diane P

Diane P is a 41 year old woman who presents with fatigue which is worse at the end of a working day. She had been noted to be mildly anaemic two years previously. The results of her FBC are Hb 110, MCV 102 fL, reticulocyte count 2.3%, WCC 3.1 x 10^3 and platelet count 133 x 10^3.[20]

What examinations should be performed?

Because of the long standing nature of the problem and the presence of pancytopenia, Diane should have a complete work-up for anaemia. The clinical examination should include assessment of potential sites of infection, lymph nodes, heart, lung, spleen, liver, vibration and 2-point discrimination particularly in the lower limbs.

What tests should be ordered?

In this case, a full range of investigations for anaemia was ordered, including B12, folate, methylmalonic acid, direct Coombs test, haptoglobin, sucrose haemolysis test, thyroid function test, electrophoresis, liver function test including bilirubin levels, HIV testing, erythrocyte sedimentation rate, and faecal occult blood testing.[20] All tests were normal, although the haptoglobin was at the lower end of the normal range (29 mg/dL). Haptoglobin tests for haemolysis, but lacks both sensitivity and specificity. Diane also had a bone marrow aspirate which showed euthyroid hyperplasia, stainable iron, no ringed sideroblasts but moderate megaloblastic changes and occasional dysplastic cells.

What is the most likely diagnosis at this stage?

Based on the results of the bone marrow aspirate, there is the possibility of a myelodysplastic syndrome. Another uncommon cause of anaemia is paroxysmal nocturnal haemoglobinuria, but both Ham's (acidified serum) test and sucrose haemolysis test were normal. Diane was placed on a trial of oral iron therapy, but her haemoglobin level remained at 115 after two months of therapy.

Because of the lack of a clear diagnosis, the patient's history and examination were reviewed. At this point it was discovered that Diane ran 8 km every second day. The conclusion was that the mild persistent anaemia was due to runner's anaemia. This is an uncommon diagnosis, thought to be due to the pounding of feet on pavement resulting in mild haemolysis and plasma volume expansion. It can also result in haemoglobinuria. The expansion in plasma volume was confirmed by measurements using radiolabelled red blood cells and radio-iodinated albumin. The anaemia is due to a dilutional effect. It is unlikely to be the cause of Diane's symptoms.

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