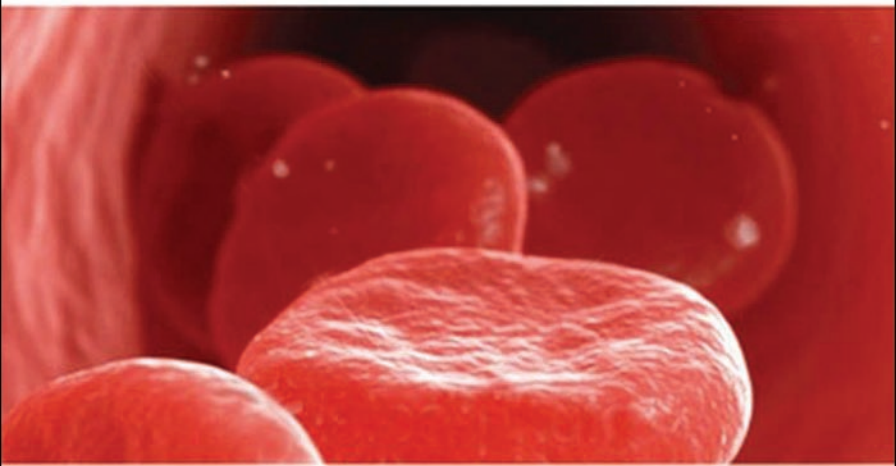
ABC of Clinical Haematology

THIRD EDITION

Edited by Drew Provan



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Third Edition

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EDITED BY

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Preface to Third Edition

In the three years since the second edition of the *ABC of Clinical Haematology*, there have been further advances in our understanding and therapies for many haematological diseases. For some disorders new classification systems have been devised. For many disorders molecular techniques have provided major diagnostic tools and greater insight into the pathogenesis of the diseases themselves.

However, despite the complexity of modern clinical haematology, the aim of the *ABC of Clinical Haematology* is to provide an overview of each disease area, with each chapter written by recognized experts in their respective areas. The structure of the book remains true to the original ABC ethos with succinct text and the liberal use of illustrations and photographic material. Key references providing more detailed information can be found at the end of each chapter to assist readers who may wish to obtain more detailed information about particular topics.

The topics covered are similar to previous editions but several chapters have been rewritten by new authors. These include myelodysplasia, leukaemias and transplantation, lymphomas, as well as bleeding disorders. Other chapters have been extensively overhauled and new co-authors included to ensure that the content is fresh and up to date. The book should be of value to a wide variety of readers including medical students, nurses, family doctors, and other health professionals involved in the care of patients with haematological disorders.

Of course, the quality of the content and the writing are key factors in the success of a book such as this and I am indebted to my haematology colleagues who have contributed high quality chapters to the book.

I would also like to thank Eleanor Lines and Vicki Donald for their help and patience during the preparation of the material.

There may be errors or omissions, and I would welcome any comments concerning the book. Readers may also have suggestions for the next edition. I would very much like to hear these and can be contacted at a.b.provan@qmul.ac.uk.

Drew Provan

CHAPTER 1

Iron Deficiency Anaemia

Drew Provan

OVERVIEW

- Iron deficiency is the commonest cause of anaemia worldwide
- Iron deficiency is usually easily diagnosed from the red cell indices
- A drop in haemoglobin is generally a late feature of iron deficiency
- The serum ferritin is a reliable means of confirming the diagnosis but may be falsely normal or even elevated as a reactive phenomenon as ferritin is an acute phase protein
- Iron deficiency is not a diagnosis in itself and in males and postmenopausal women blood loss from the gastrointestinal tract must be excluded
- Oral iron is preferred for iron replacement therapy, but occasionally parenteral iron is required

Box 1.1 **Risk factors for development of iron deficiency**

- **Age:** infants (especially if there is a history of prematurity); adolescents; postmenopausal women; elderly people
- **Sex:** increased risk in women
- **Reproduction:** menorrhagia
- **Renal:** haematuria (rarer cause)
- **Gastrointestinal tract:** appetite or weight changes; changes in bowel habit; bleeding from rectum/melaena; gastric or bowel surgery
- **Drug history:** especially aspirin and non-steroidal anti-inflammatories
- **Social history:** diet, especially vegetarians
- **Physiological:** pregnancy; infancy; adolescence; breastfeeding; age of weaning

Iron deficiency is the commonest cause of anaemia worldwide and is frequently seen in general practice. Iron deficiency anaemia is caused by defective synthesis of haemoglobin, resulting in red cells that are smaller than normal (microcytic) and contain reduced amounts of haemoglobin (hypochromic).

Iron metabolism

Iron has a pivotal role in many metabolic processes, and the average adult contains 3–5 g of iron, of which two-thirds is in the oxygen-carrying molecule haemoglobin.

A normal Western diet provides about 15 mg of iron daily, of which 5–10% is absorbed (~ 1 mg), principally in the duodenum and upper jejunum, where the acidic conditions help the absorption of iron in the ferrous form. Absorption is helped by the presence of other reducing substances, such as hydrochloric acid and ascorbic acid. The body has the capacity to increase its iron absorption in the face of increased demand, for example, in pregnancy, lactation, growth spurts and iron deficiency (Box 1.1).

Once absorbed from the bowel, iron is transported across the mucosal cell to the blood, where it is carried by the protein transferrin to developing red cells in the bone marrow. Iron stores comprise ferritin, a labile and readily accessible source of iron and haemosiderin, an insoluble form found predominantly in macrophages.

About 1 mg of iron a day is shed from the body in urine, faeces, sweat and cells shed from the skin and gastrointestinal tract. Menstrual losses of an additional 20 mg per month and the increased requirements of pregnancy (500–1000 mg) contribute to the higher incidence of iron deficiency in women of reproductive age (Table 1.1, Box 1.2).

Clinical features of iron deficiency

The symptoms accompanying iron deficiency depend on how rapidly the anaemia develops. In cases of chronic, slow blood loss, the body adapts to the increasing anaemia and patients can often tolerate extremely low concentrations of haemoglobin, for example, < 7.0 g/dL, with remarkably few symptoms. Most patients complain of increasing lethargy and dyspnoea. More unusual symptoms are headaches, tinnitus and taste disturbance.

Table 1.1 Daily dietary iron requirements

	Amount (mg)
Male	1
Adolescence	2–3
Female (reproductive age)	2–3
Pregnancy	3–4
Infancy	1
Maximum bioavailability from normal diet	~4

Box 1.2 **Causes of iron deficiency anaemia**

Reproductive system
- Menorrhagia

Gastrointestinal tract

Bleeding
- Oesophagitis
- Oesophageal varices
- Hiatus hernia (ulcerated)
- Peptic ulcer
- Inflammatory bowel disease
- Haemorrhoids (rarely)
- Carcinoma: stomach, colorectal
- Angiodysplasia
- Hereditary haemorrhagic telangiectasia (rare)

Malabsorption
- Coeliac disease
- Atrophic gastritis (also may result from iron deficiency)

Physiological
- Growth spurts (especially in premature infants)
- Pregnancy

Dietary
- Vegans
- Elderly
- Other
- Patients with chronic renal failure undergoing haemodialysis and receiving erythropoietin

Worldwide commonest cause of iron deficiency is hookworm infection

On examination, several skin, nail and other epithelial changes may be seen in chronic iron deficiency. Atrophy of the skin occurs in about a third of patients and (rarely nowadays) nail changes such as koilonychia (spoon-shaped nails; Fig. 1.1) may result in brittle, flattened nails. Patients may also complain of angular stomatitis, in which painful cracks appear at the angle of the mouth, sometimes accompanied by glossitis. Although uncommon, oesophageal and pharyngeal webs can be a feature of iron deficiency anaemia (consider this in middle aged women presenting with dysphagia). These changes are believed to be due to a reduction in the iron-containing enzymes in the epithelium and gastrointestinal tract. Few of these epithelial changes are seen in modern practice, and they are of limited diagnostic value.

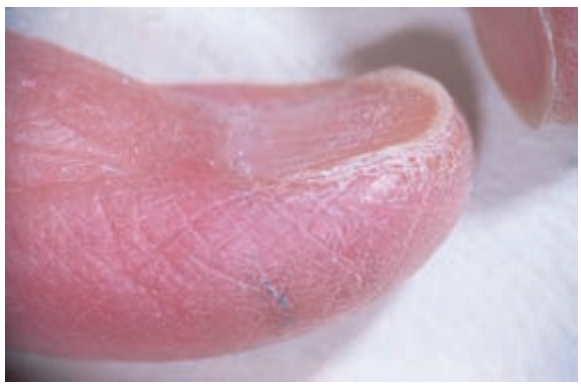

Figure 1.1 Nail changes in iron deficiency anaemia (koilonychia).

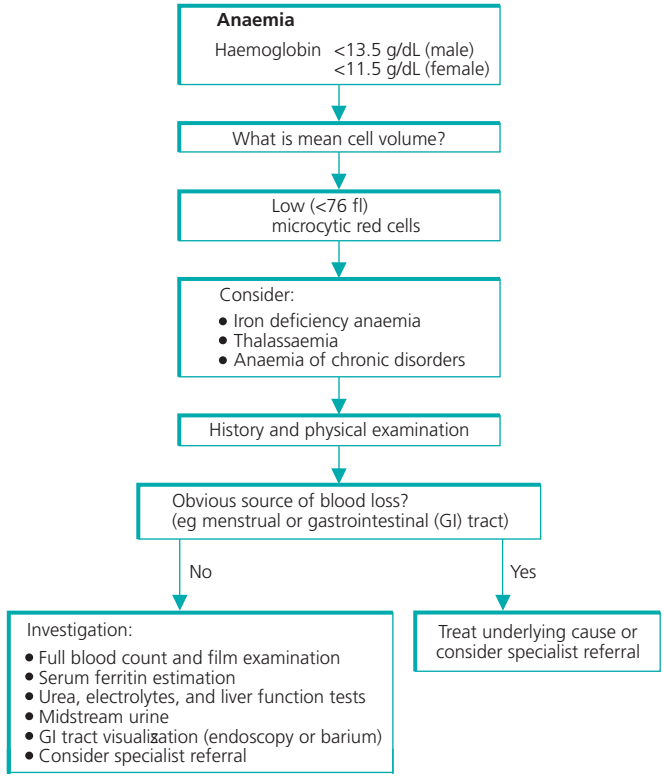

Figure 1.2 Diagnosis and investigation of iron deficiency anaemia.

Tachycardia and cardiac failure may occur with severe anaemia irrespective of cause and, in such cases, prompt remedial action should be taken.

When iron deficiency is confirmed, a full clinical history, including leading questions on possible gastrointestinal blood loss or malabsorption (as in, for example, coeliac disease), should be obtained. Menstrual losses should be assessed and the importance of dietary factors and regular blood donation should not be overlooked (Fig. 1.2).

Diet alone is seldom the sole cause of iron deficiency anaemia in the UK except when it prevents an adequate response to a physiological challenge, as in pregnancy, for example.

Laboratory investigations

A full blood count and film should be assessed (Box 1.3). These will confirm the anaemia; recognizing the indices of iron deficiency is usually straightforward (reduced haemoglobin concentration, reduced mean cell volume, reduced mean cell haemoglobin, reduced mean cell haemoglobin concentration) (Table 1.2). Some modern analysers will determine the percentage of hypochromic red cells, which may be high before the anaemia develops (it is worth noting that a reduction in haemoglobin concentration is a late feature of iron deficiency). The blood film shows microcytic hypochromic red cells (Fig. 1.3). Hypochromic anaemia occurs in other disorders, such as anaemia of chronic disorders and sideroblastic anaemias,

Box 1.3 **Investigations in iron deficiency anaemia**

- Full clinical history and physical examination
- Full blood count and blood film examination
- Haematinic assays (serum ferritin, vitamin B_{12}, folate)
- Note serum iron and TIBC now obsolete
- Percentage of hypochromic red cells and soluble transferrin receptor assay (if available)
- Urea and electrolytes, liver function tests
- Fibreoptic and/or barium studies of the gastrointestinal tract
- Pelvic ultrasound (female patients, if indicated)

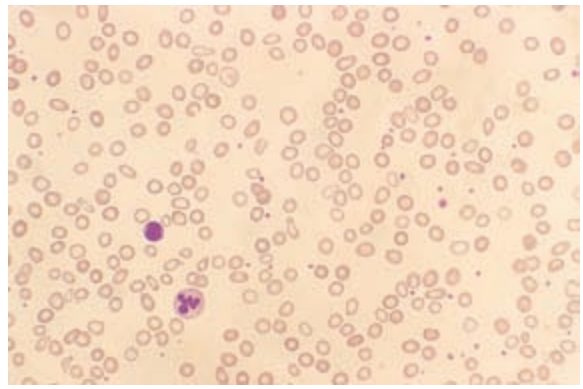

Figure 1.3 Blood film showing changes of iron deficiency anaemia.

and in globin synthesis disorders, such as thalassaemia (Table 1.3). To help to differentiate the type, further haematinic assays may be necessary. Historically, serum iron and total iron binding capacity (TIBC) were used in the diagnosis of iron deficiency anaemia, but because of the wide diurnal variation seen in iron levels and the lack of sensitivity, these assays are seldom used today. Difficulties in diagnosis arise when more than one type of anaemia is present, for example, iron deficiency and folate deficiency in malabsorption, in a population where thalassaemia is present, or in pregnancy, when the interpretation of red cell indices may be difficult.

Haematinic assays will demonstrate reduced serum ferritin concentration in straightforward iron deficiency. As an acute phase protein, however, the serum ferritin concentration may be normal or even raised in inflammatory or malignant disease.

A prime example of this is found in rheumatoid disease, in which active disease may result in a spuriously raised serum ferritin concentration masking an underlying iron deficiency caused by gastrointestinal bleeding after non-steroidal analgesic treatment. There may also be confusion in liver disease, as the liver contains stores of ferritin that are released after hepatocellular damage, leading to raised serum ferritin concentrations. In cases where ferritin estimation is likely to be misleading, the soluble transferrin receptor (sTfR) assay may aid the diagnosis.

Transferrin receptors are found on the surface of red cells in greater numbers in iron deficiency; a proportion of receptors is shed into the plasma and can be measured using commercial kits. Unlike serum ferritin, the level of sTfR does not rise in inflammatory disorders, and

Table 1.2 Diagnosis of iron deficiency anaemia

Reduced haemoglobin	Men <13.5 g/dl, women <11.5 g/dl
Reduced MCV	<76 fl (76–95 fl)
Reduced MCH	29.5 ± 2.5 pg (27.0–32.0 pg)
Reduced MCHC	32.5 ± 2.5 g/dl (32.0–36.0 g/dl)
Blood film	Microcytic hypochromic red cells with pencil cells and target cells
Reduced serum ferritin*	Men <10 µg/L, women (postmenopausal) <10 µg/L (premenopausal) <5µg/L
Elevated % hypochromic red cells (>2%)	
Elevated soluble transferrin receptor level	

*Check with local laboratory for reference ranges. Note normal values in parentheses
MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume

Table 1.3 Characteristics of anaemia associated with other disorders

	Iron deficiency	**Chronic disorders**	**Thalassaemia trait (α or β)**	**Sideroblastic anaemia**
Degree of anaemia	Any	Seldom <9.0 g/dl	Mild	Any
MCV	↓	N or ↓	↓↓	N or ↓ or ↑
Serum ferritin	↓	N or ↑	N	↑
sTfR	↑	N	↑	N
Marrow iron	Absent	Present	Present	Present

MCV, mean corpuscular volume; N, normal; sTfR, soluble transferrin receptor assay

hence can help to differentiate between anaemia due to inflammation and iron deficiency.

Diagnostic bone marrow sampling is seldom performed in simple iron deficiency, but, if the diagnosis is in doubt, a marrow aspirate may be carried out to demonstrate absent bone marrow stores.

When iron deficiency has been diagnosed, the underlying cause should be investigated and treated. Often the history will indicate the likely source of bleeding, for example, menstrual blood loss or gastrointestinal bleeding. If there is no obvious cause, further investigation generally depends on the age and sex of the patient. In male patients and postmenopausal women, possible gastrointestinal blood loss is investigated by visualization of the gastrointestinal tract (endoscopic or barium studies). Faecal occult blood tests are of no value in the investigation of iron deficiency.

Management

Effective management of iron deficiency relies on (i) the appropriate management of the underlying cause (for example, gastrointestinal or menstrual blood loss) and (ii) iron replacement therapy.

Oral iron replacement therapy, with gradual replenishment of iron stores and restoration of haemoglobin, is the preferred treatment. Oral ferrous salts are the treatment of choice (ferric salts are less well absorbed) and usually take the form of ferrous sulphate 200 mg three times daily (providing 65 mg × 3 = 195 mg elemental iron/day) (Fig. 1.4). Alternative preparations include ferrous gluconate and ferrous fumarate (Table 1.4). All three compounds, however, are associated with a high incidence of side effects, including nausea, constipation and diarrhoea. These side effects may be reduced by taking the tablets after meals, but even milder symptoms account for poor compliance with oral iron supplementation. It is worth noting that these lower gastrointestinal symptoms are not dose related. Modified release preparations have been developed to reduce side effects, but in practice prove expensive and often release the iron beyond the sites of optimal absorption.

Figure 1.4 Oral iron replacement therapy.

Table 1.4 Elemental iron content of various oral iron preparations

Preparation	Amount (mg)	Ferrous iron (mg)
Ferrous fumarate	200	65
Ferrous gluconate	300	35
Ferrous sulphate	300	60

Effective iron replacement therapy should result in a rise in haemoglobin concentration of around 0.1 g/dL per day (about 2 g/dL every 3 weeks), but this varies from patient to patient. Once the haemoglobin concentration is within the normal range, iron replacement should continue for 3 months to replenish the iron stores.

Failure to respond to oral iron therapy

The main reason for failure to respond to oral iron therapy is poor compliance. However, if the losses (for example, bleeding) exceed the amount of iron absorbed daily, the haemoglobin concentration will not rise as expected; this will also be the case in combined deficiency states.

The presence of underlying inflammation or malignancy may also lead to a poor response to therapy. Occasionally, malabsorption of iron, such as that seen in coeliac disease, may lead to a failure to respond. Finally, an incorrect diagnosis of iron deficiency anaemia should be considered in patients who fail to respond adequately to iron replacement therapy.

Intravenous and intramuscular iron preparations

Parenteral iron may be used when the patient cannot tolerate oral supplements, for example, when patients have severe gastrointestinal side effects or if the losses exceed the daily amount that can be absorbed orally (Box 1.4). The rise in haemoglobin concentration is no faster with parenteral iron preparations than with oral iron therapy.

Intramuscular iron sorbitol (a complex of iron, sorbitol and citric acid) injection was used as a parenteral iron replacement for many years, but was discontinued in the UK in 2003. Generally, around 10–20 deep intramuscular injections were given over 2–3·weeks. However, side effects were common and included pain, skin staining at the site of injection and arthralgia. Newer intravenous iron preparations include iron hydroxide sucrose (Venofer®) and iron dextran (Cosmofer ®, may also be given IM) for use in selected cases and under strict medical supervision, for example, on a haematology day unit (risk of anaphylaxis or other reactions).

Alternative treatments

Blood transfusion is not indicated unless the patient has decompensated due to a drop in haemoglobin concentration and needs a more rapid rise in haemoglobin, for example, in cases of worsening angina or severe coexisting pulmonary disease. In cases of iron deficiency with serious ongoing acute bleeding, blood transfusion may be required.

Prevention

When absorption from the diet is likely to be matched or exceeded by losses, extra sources of iron should be considered, for example,

Box 1.4 **Intravenous iron preparations**

- Intramuscular iron sorbitol no longer available (severe reactions)
- Iron hydroxide sucrose and iron dextran are currently available in the UK
- Useful in selected cases
- Must be given under close medical supervision and where full resuscitation facilities are available
- A test dose is recommended before administration of the full dose

prophylactic iron supplements in pregnancy or after gastrectomy, or encouragement of breastfeeding or use of formula milk during the first year of life (rather than cows' milk, which is a poor source of iron).

Further reading

Baer AN, Dessypris EN, Krantz SB. The pathogenesis of anemia in rheumatoid arthritis: a clinical and laboratory analysis. *Seminars in Arthritis and Rheumatism* 1990; **19**: 209–23.

Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. *Clinica Chimica Acta* 2003; **329**: 9–22.

Cook JD. Diagnosis and management of iron deficiencyanaemia. *Balliere's Best Practice and Research. Clinical Haematology* 2005; **18**: 319–32.

Cook JD, Skikne BS, Baynes RD. Iron deficiency: the global perspective. *Advances in Experimental Medicine and Biology* 1994; **356**: 219–28.

DeMaeyer E, Adiels-Tegman M. The prevalence of anaemia in the world. *World Health Statistics Quarterly* 1985; **38**: 302–16.

Demir A, Yarali N, Fisgin T *et al.* Serum transferrin receptor levels in beta-thalassemia trait. *Journal of Tropical Pediatrics* 2004; **50**: 369–71.

Ferguson BJ, Skikne BS, Simpson KM *et al.* Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. *Journal of Laboratory and Clinical Medicine* 1992; **119**: 385–90.

Lozoff B, De Andraca I, Castillo M *et al.* Behavioral and developmental effects of preventing iron deficiency anemia in healthy full-term infants. *Pediatrics* 2003; **112**: 846–54.

McIntyre AS, Long RG. Prospective survey of investigations in outpatients referred with iron deficiency anaemia. *Gut* 1993; **34**: 1102–7.

Provan D. Mechanisms and management of iron deficiency anaemia. *British Journal of Haematology* 1999; **105**(Suppl 1): 19–26.

Punnonen K, Irjala K, Rajamaki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood* 1997; **89**: 1052–7.

Rockey DC, Cello JP. Evaluation of the gastrointestinal tract in patients with iron deficiency anemia. *New England Journal of Medicine* 1993; **329**: 1691–5.

Windsor CW, Collis JL. Anaemia and hiatus hernia: experience in 450 patients. *Thorax* 1967; **22**(1): 73–8.

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CHAPTER 2

Macrocytic Anaemias

A Victor Hoffbrand, Drew Provan

OVERVIEW

- Macrocytic red cells (MCV greater than 95fl) may be associated with a megaloblastic or normoblastic bone marrow
- Deficiencies of either vitamin B_{12} or folate lead to defective DNA synthesis, megaloblastic changes in the bone marrow and many other cells
- The blood count indices and blood film features of B_{12} and folate deficiencies are identical and specific haematinic assays are required to differentiate between them
- Pernicious anaemia is the commonest cause of B_{12} deficiency in the UK
- Folate deficiency occurs in pregnancy, prematurity, chronic haemolysis and other high cell turnover states
- Vitamin B_{12} deficiency may lead to progressive neuropathy even in the absence of anaemia

Macrocytosis is a rise in the mean cell volume (MCV) of red cells above the normal range (in adults 80–95 fl). It is detected using a blood count, in which the MCV and other red cell indices are measured. The MCV is lower in children than in adults, with a normal mean of 70 fl at 1 year of age, rising by about 1 fl each year until it reaches the adult volume at puberty.

The causes of macrocytosis fall into two groups: (i) deficiency of vitamin B_{12} (cobalamin) or folate (or rarely abnormalities of their metabolism), in which the bone marrow is megaloblastic (Box 2.1) and (ii) other causes (Box 2.2), in which the bone marrow is usually normoblastic. In this chapter, the two groups are considered separately. The steps to diagnose the cause of macrocytosis and subsequently to manage it are then considered.

Megaloblastic bone marrow

Megaloblastic bone marrow is exemplified by developing red blood cells that are larger than normal, with nuclei that are more immature than the cytoplasm. The underlying mechanism is defective DNA synthesis.

Defects of vitamin B_{12} metabolism, for example, transcobalamin II deficiency, nitrous oxide anaesthesia, or of folate metabolism (such as methotrexate treatment), or rare inherited defects of DNA synthesis, may all cause megaloblastic anaemia.

Box 2.1 **Causes of megaloblastic anaemia**

Diet
- Vitamin B_{12} deficiency: vegan diet, poor quality diet
- Folate deficiency: poor quality diet, old age, poverty, synthetic diet without added folic acid, goats' milk

Malabsorption
- Gastric causes of vitamin B_{12} deficiency: pernicious anaemia, congenital intrinsic factor deficiency or abnormality, gastrectomy
- Intestinal causes of vitamin B_{12} deficiency: stagnant loop, congenital selective malabsorption, ileal resection, Crohn's disease
- Intestinal causes of folate deficiency: coeliac disease, tropical sprue, jejunal resection

Increased cell turnover
- Folate deficiency: pregnancy, prematurity, chronic haemolytic anaemia (such as sickle cell anaemia), extensive inflammatory and malignant diseases

Renal loss
- Folate deficiency: congestive cardiac failure, dialysis

Drugs
- Folate deficiency: anticonvulsants, sulphasalazine

Box 2.2 **Other causes of macrocytosis***

- Alcohol
- Myelodysplasia
- Liver disease
- Cytotoxic drugs
- Hypothyroidism
- Paraproteinaemia (such as myeloma)
- Reticulocytosis
- Pregnancy
- Aplastic anaemia
- Neonatal period
- Red cell aplasia

*These are usually associated with a normoblastic marrow

Deficiency of vitamin B_{12} or folate

Vitamin B_{12} deficiency

The body's requirement for vitamin B_{12} is about 1 µg daily. This is

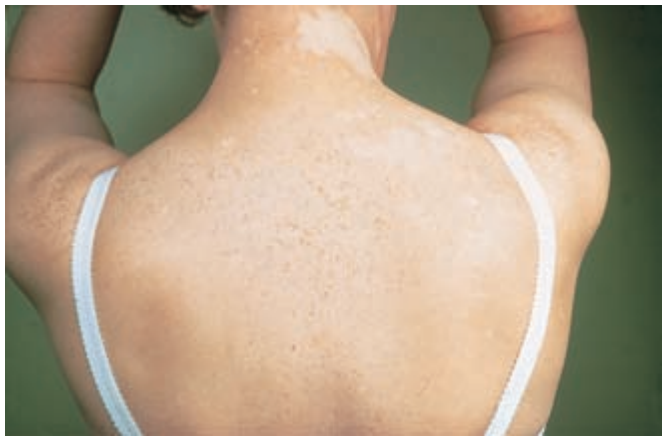

Figure 2.1 Patient with vitiligo on neck and back.

amply supplied by a normal Western diet (vitamin B_{12} content 10–30 μg daily) but not by a strict vegan diet, which excludes all animal produce (including milk, eggs and cheese). Absorption of vitamin B_{12} is through the ileum, facilitated by intrinsic factor, which is secreted by the parietal cells of the stomach. Absorption by this mechanism is limited to 2–3 μg daily.

In Britain, vitamin B_{12} deficiency is usually due to pernicious anaemia, which now accounts for up to 80% of all cases of megaloblastic anaemia. The incidence of the disease is 1:10 000 in northern Europe and the disease occurs in all races. The underlying mechanism is an autoimmune gastritis that results in achlorhydria and the absence of intrinsic factor. The incidence of pernicious anaemia peaks at 60 years of age; the condition has a female : male incidence of 1.6 : 1.0 and is more common in those with early greying of hair, blue eyes, blood group A and in those with a family history of pernicious anaemia or associated diseases, for example, vitiligo (Fig. 2.1), myxoedema, Hashimoto's disease, Addison's disease and hypoparathyroidism.

Other causes of vitamin B_{12} deficiency are infrequent in the UK. A vegan lifestyle is an unusual cause of severe deficiency, as most vegetarians and vegans include some vitamin B_{12} in their diet. Moreover, unlike in pernicious anaemia, the enterohepatic circulation for vitamin B_{12} is intact in vegans, so vitamin B_{12} stores are conserved. Gastric resection and intestinal causes of malabsorption of vitamin B_{12}, for example, ileal resection or the intestinal stagnant loop syndrome, are less common now that abdominal tuberculosis is infrequent and H2 antagonists have been introduced for treating peptic ulceration, thus reducing the need for gastrectomy.

Folate deficiency

The daily requirement for folate is 100–200 μg and a normal mixed diet contains about 200–300 μg. Natural folates are largely found in the polyglutamate form and these are absorbed through the upper small intestine after deconjugation and conversion to the monoglutamate 5-methyltetrahydrofolate.

Body stores are sufficient for only about 4 months. Folate deficiency may arise because of inadequate dietary intake, malabsorption (especially coeliac disease; Fig. 2.2), or excessive use caused by proliferating cells, which degrade folate. Deficiency in pregnancy may be due partly to inadequate diet, partly to transfer of folate to the fetus and partly to increased folate degradation.

Figure 2.2 Patient with coeliac disease: underweight and low stature.

Consequences of vitamin B_{12} or folate deficiency

Megaloblastic anaemia

Clinical features include pallor and jaundice. The onset is gradual, and a severely anaemic patient may present with congestive heart failure or only when an infection supervenes. The blood film shows oval macrocytes and hypersegmented neutrophil nuclei (with six or more lobes) (Fig. 2.3). In severe cases, the white cell count and platelet count also fall (pancytopenia). The bone marrow shows characteristic megaloblastic erythroblasts and giant metamyelocytes (granulocyte precur-

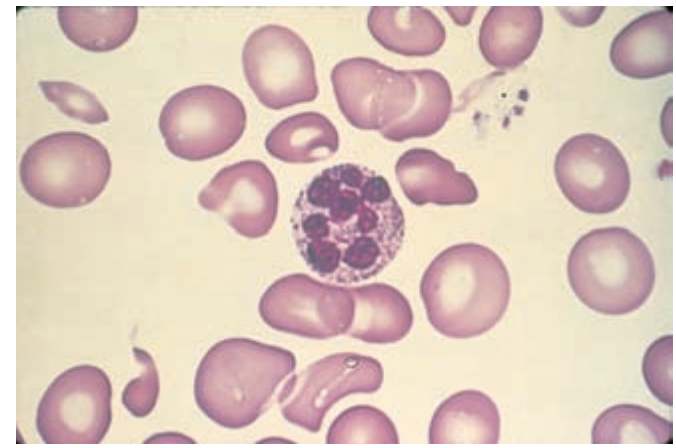

Figure 2.3 Blood film in vitamin B_{12} deficiency showing macrocytic red cells and a hypersegmented neutrophil.

sors). Biochemically, there is an increase of unconjugated bilirubin and serum lactic dehydrogenase in the plasma, with, in severe cases, an absence of haptoglobins and presence of haemosiderin in the urine. These changes, including jaundice, are due to increased destruction of red cell precursors in the marrow (ineffective erythropoiesis).

Vitamin B_{12} neuropathy

A minority of patients with vitamin B_{12} deficiency develop a neuropathy due to symmetrical damage to the peripheral nerves and posterior and lateral columns of the spinal cord, the legs being more affected than the arms. Psychiatric abnormalities and visual disturbance may also occur. Men are more commonly affected than women. The neuropathy may occur in the absence of anaemia. Psychiatric changes and, at most, a mild peripheral neuropathy may be ascribed to folate deficiency.

Neural tube defects

Folic acid supplements in pregnancy have been shown to reduce the incidence of neural tube defects (spina bifida, encephalocoele and anencephaly) in the fetus, and may also reduce the incidence of cleft palate and harelip (Box 2.3). No clear relation exists between the incidence of these defects and any folate deficiency in the mother, although the lower the maternal red cell folate (and serum vitamin B_{12}) concentrations, even within the normal range, the more likely neural tube defects are to occur in the fetus. An underlying mechanism in a minority of cases is a genetic defect in folate metabolism, a mutation in the enzyme 5,10-methylene-tetrahydrofolate reductase. An autoantibody to folate receptors has been detected in pregnancy in some women who have babies with neural tube defects.

Gonadal dysfunction

Deficiency of either vitamin B_{12} or folate may cause sterility, which is reversible with appropriate vitamin supplementation.

Epithelial cell changes

Glossitis may occur, and other epithelial surfaces may show cytological abnormalities (Fig. 2.4).

Box 2.3 **Preventing folate deficiency in pregnancy**

- As prophylaxis against folate deficiency in pregnancy, daily doses of folic acid 400 µg are usual
- Larger doses are not recommended as they could theoretically mask megaloblastic anaemia due to vitamin B_{12} deficiency and thus allow B_{12} neuropathy to develop
- As neural tube defects occur by the 28th day of pregnancy, it is advisable for a woman's daily folate intake to be increased by 400 µg/day at the time of conception
- The US Food and Drugs Administration announced in 1996 that specified grain products (including most enriched breads, flours, cornmeal, rice, noodles and macaroni) will be required to be fortified with folic acid to levels ranging from 0.43 mg to 1.5 mg per pound (453 g) of product. Fortification of flour with folic acid is currently under discussion in the UK
- For mothers who have already had an infant with a neural tube defect, larger doses of folic acid, for example, 5 mg daily, are recommended before and during subsequent pregnancy

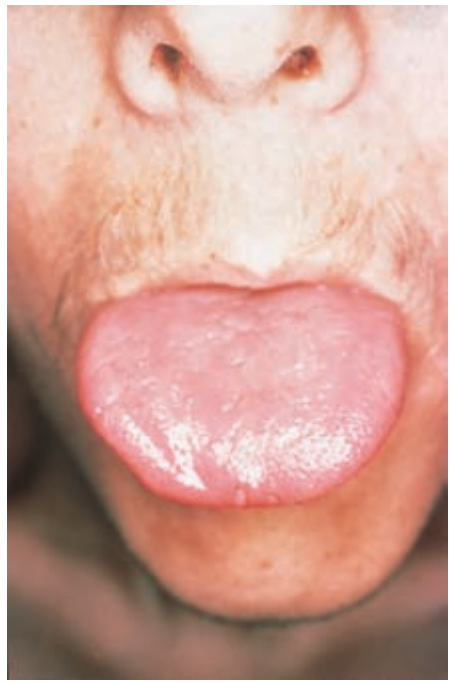

Figure 2.4 Glossitis due to vitamin B_{12} deficiency.

Cardiovascular disease

Raised serum homocysteine concentrations have been associated with arterial obstruction (myocardial infarct, peripheral vascular disease or stroke) and venous thrombosis. Trials are under way to determine whether folic acid supplementation reduces the incidence of these vascular diseases.

Other causes of macrocytosis

The most common cause of macrocytosis in the UK is alcohol. Fairly small quantities of alcohol, for example, two gin and tonics or half a bottle of wine a day, especially in women, may cause a rise of MCV to > 100 fl, typically without anaemia or any detectable change in liver function.

The mechanism for the rise in MCV is uncertain. In liver disease, the red cell volume may rise as a result of excessive lipid deposition on red cell membranes, and the rise is particularly pronounced in liver disease caused by alcohol. A modest rise in MCV is found in severe thyroid deficiency.

Physiological causes of macrocytosis are pregnancy and the neonatal period. In other causes of macrocytosis, other haematological abnormalities are usually present; in myelodysplasia (a frequent cause of macrocytosis in elderly people), there are usually quantitative or qualitative changes in the white cells and platelets in the blood. In aplastic anaemia, pancytopenia is present; pure red cell aplasia may also cause macrocytosis. Changes in plasma proteins, for example, presence of a paraprotein (as in myeloma), may cause a rise in MCV without macrocytes being present in the blood film. Drugs that affect DNA synthesis, for example, hydroxyurea and azathioprine, can cause macrocytosis with or without megaloblastic changes. Finally, a rare, benign familial type of macrocytosis has been described.

Diagnosis

Biochemical assays

The most widely used screening tests for the deficiencies are the serum vitamin B_{12} and folate assays (Box 2.4). A low serum concentration implies deficiency, but a subnormal serum concentration may occur in the absence of pronounced body deficiency, for example, in pregnancy (vitamin B_{12}) and with recent poor dietary intake (folate).

Red cell folate can also be used to screen for folate deficiency; a low concentration usually implies appreciable depletion of body folate, but the concentration also falls in severe vitamin B_{12} deficiency, and so it is more difficult to interpret the significance of a low red cell count than serum folate concentration in patients with megaloblastic anaemia. Moreover, if the patient has received a recent blood transfusion, the red cell folate concentration will partly reflect the folate concentration of the transfused red cells.

Specialist investigations

Assays of serum homocysteine (raised in vitamin B_{12} or folate deficiency) or methylmalonic acid (raised in vitamin B_{12} deficiency) are used in some specialized laboratories. Serum homocysteine levels are also raised in renal failure and with certain drugs, such as corticosteroids, and they increase with age and smoking.

Autoantibodies

For patients with vitamin B_{12} or folate deficiency, it is important to establish the underlying cause. In pernicious anaemia, intrinsic factor antibodies are present in plasma in 50% of patients and in parietal cell antibodies in 90%. Antiendomysial and antitransglutaminase antibodies are usually positive in coeliac disease.

Other investigations

A bone marrow examination is usually performed to confirm megaloblastic anaemia (Fig. 2.5). It is also required for the diagnosis of myelodysplasia (Fig. 2.6), aplastic anaemia, myeloma, or other marrow disorders associated with macrocytosis.

Box 2.4 **Investigations that may be needed in patients with macrocytosis**

- Serum vitamin B_{12} assay
- Serum and red cell folate assays
- Liver and thyroid function
- Reticulocyte count
- Serum protein electrophoresis
- For vitamin B_{12} deficiency: serum parietal cell and intrinsic factor antibodies, radioactive vitamin B_{12} absorption with and without intrinsic factor (Schilling's test), possibly serum gastrin concentration
- For folate deficiency: antiendomysial and antitransglutaminase antibodies
- Consider bone marrow examination for megaloblastic changes suggestive of vitamin B_{12} or folate deficiency, or alternative diagnoses, for example, myelodysplasia, aplastic anaemia, myeloma
- Endoscopy: gastric biopsy (vitamin B_{12} deficiency); duodenal biopsy (folate deficiency)

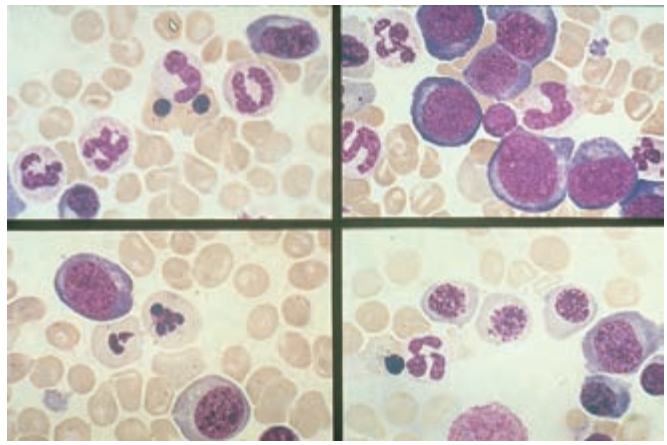

Figure 2.5 Bone marrow appearances in megaloblastic anaemia: developing red cells are larger than normal, with nuclei that are immature relative to their cytoplasm (nuclear : cytoplasmic asynchrony).

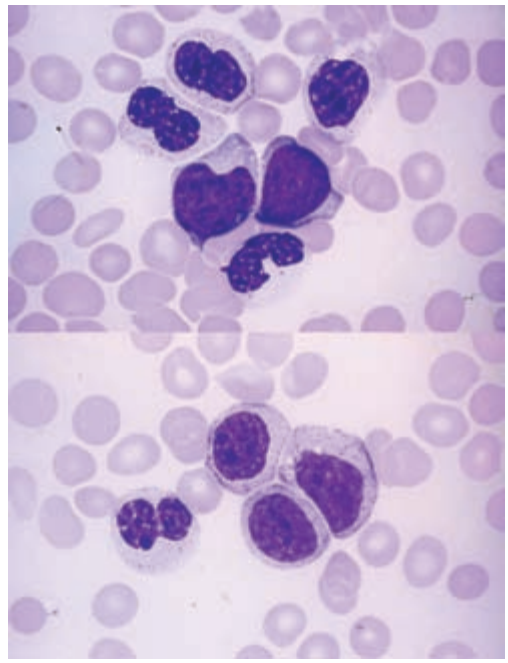

Figure 2.6 Bone marrow aspirate in myelodysplasia showing characteristic dysplastic neutrophils with bilobed nuclei. Reproduced with permission from *Clinical Haematology* (AV Hoffbrand, J Pettit), 3rd edn. St Louis: CV Mosby, 2000.

Radioactive vitamin B_{12} absorption studies, for example, Schilling's test, show impaired absorption of the vitamin in pernicious anaemia (Table 2.1); this can be corrected by giving intrinsic factor. In patients with an intestinal lesion, however, absorption of vitamin B_{12} cannot be corrected with intrinsic factor. Human intrinsic factor is no longer licensed for this test because of concern about transmission of prion disease.

Endoscopy should be performed to confirm atrophic gastritis and exclude gastric carcinoma or gastric polyps, which are 2–3 times more common in patients with pernicious anaemia than in age- and sex-matched controls.

Table 2.1 Results of absorption tests of radioactive vitamin B_{12}

	Dose of vitamin B_{12} given alone	Dose of vitamin B_{12} given with intrinsic factor†
Vegan	Normal	Normal
Pernicious anaemia or gastrectomy	Low	Normal
Ileal resection	Low	Low
Intestinal blind loop syndrome	Low*	Low*

*Corrected by antibiotics.
†Human intrinsic factor no longer licensed for this test because of concern about prion transmission

If folate deficiency is diagnosed, it is important to assess dietary folate intake and to exclude coeliac disease by tests for serum antiendomysial and antitransglutaminase antibodies, endoscopy and duodenal biopsy. The deficiency is common in patients with diseases of increased cell turnover who also have a poor diet.

Treatment

Vitamin B_{12} deficiency is treated initially by giving the patient six injections of hydroxocobalamin 1 mg at intervals of about 3–4 days, followed by four such injections a year for life. For patients undergoing total gastrectomy or ileal resection, it is sensible to start the maintenance injections from the time of operation. For vegans, less frequent injections, for example, 1–2 per year, may be sufficient, and the patient should be advised to eat foods to which vitamin B_{12} has been added, such as certain fortified breads or other foods.

Folate deficiency is treated with folic acid, usually 5 mg daily orally for 4 months, which is continued only if the underlying cause cannot be corrected. As prophylaxis against folate deficiency in patients with a severe haemolytic anaemia, such as sickle cell anaemia, 5 mg folic acid once weekly is probably sufficient. Vitamin B_{12} deficiency must be excluded in all patients starting folic acid treatment at these doses, as such treatment may correct the anaemia in vitamin B_{12} deficiency but allow neurological disease to develop.

Further reading

Carmel R. Current concepts in cobalamin deficiency. *Annual Reviews in Medicine* 2000; 51: 357–75.

Clarke R, Grimley Evans J, Schneede J *et al*. Vitamin B12 and folate deficiency in later life. *Age and Ageing* 2004; **33**: 34–41.

Hershko C, Hoffbrand AV, Keret D *et al*. Role of autoimmune gastritis, Helicobacter pylori and celiac disease in refractory or unexplained iron deficiency anemia. *Haematologica* 2005; **90**: 585–95.

Jacques PF, Selhub J, Bostom AG *et al*. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *New England Journal of Medicine* 1999; **340**: 1449–54.

Lindenbaum J, Allen RH. Clinical spectrum and diagnosis of folate deficiency. In: Bailey LB, ed. *Folate in Health and Disease*. Marcel Dekker, New York,1995, 43–73.

Mills JL. Fortification of foods with folic acid—how much is enough? *New England Journal of Medicine* 2000; **342**(19): 1442–5.

Perry DJ. Hyperhomocysteinaemia. *Bailliere's Best Practice and Research. Clinical Haematology* 1999; **12**: 451–77.

Rothenberg SP, da Costa MP, Sequeira JM *et al*. Autoantibodies against folate receptors in women with a pregnancy complicated by a neural-tube defect. *New England Journal of Medicine* 2004; **350**: 134–42.

Solomon LR. Cobalamin-responsive disorders in the ambulatory care setting: unreliability of cobalamin, methylmalonic acid, and homocysteine testing. *Blood* 2005; **105**: 978–85.

Wickramasinghe SN. Megaloblastic anaemia. *Bailliere's Clinical Haematology* 1995; **8**: 441–703.