

CSP

Common Sense Pathology

A REGULAR CASE-BASED SERIES ON PRACTICAL PATHOLOGY FOR GPs

CONTENTS

- Normal iron status
- Iron deficiency
- Case studies



Diagnosis of **IRON DEFICIENCY** and **IRON OVERLOAD**

A JOINT INITIATIVE OF



Australian
Doctor.



Australian Government
Department of Health and Ageing

Diagnosis of iron deficiency and iron overload



Dr Alan R McNeil, head, department of chemical pathology, Dorevitch Pathology, Heidelberg, Victoria.

Emeritus Professor Jack Metz, consultant haematologist, Dorevitch Pathology, Heidelberg, Victoria; and honorary consultant, department of haematology, Royal Melbourne Hospital, Victoria.

Normal iron status

Healthy adults have 3000-4000mg of iron in their body, most of which is found inside haemoglobin. Iron is efficiently recycled, so daily requirements are only 1-2mg. These requirements are increased in children and women of reproductive age, and can be five times higher during pregnancy.

Bleeding is the most common cause of increased iron losses with each 1mL of blood containing 0.5mg of iron. For example, daily iron requirements may be doubled in someone losing 80mL of menstrual blood per month or 2-3mL of blood each day from a bleeding bowel cancer.

Because most of the iron in food is not absorbed, daily dietary requirements range from 10mg to 20mg. Depending on diet, most iron comes from plant sources even though this is less well absorbed than the iron in red meat. The fraction absorbed increases in people with iron deficiency. Absorption is increased by ascorbic acid and decreased by drugs such as tetracyclines and antacids, as well as by naturally occurring iron binders in food like phytates in vegetables and tannins in tea.

Iron status can be viewed as a continuum between iron-deficiency anaemia at one end and iron overload at the other. Many people lie between these extremes with milder degrees of iron deficiency and excess. They typically have normal haematological parameters and diagnosis depends on the detection of reduced serum ferritin and increased transferrin saturation, respectively (table 1).

This issue of *Common Sense Pathology* is a joint initiative of *Australian Doctor* and the Royal College of Pathologists of Australasia.

It is published by Reed Business Information
Tower 2, 475 Victoria Ave, Locked Bag 2999
Chatswood DC NSW 2067.
Ph: (02) 9422 2999 Fax: (02) 9422 2800
E-mail: mail@australiandoctor.com.au
Web site: www.australiandoctor.com.au
(Inc. in NSW) ACN 000 146 921
ABN 47 000 146 921 ISSN 1039-7116

© 2006 by the Royal College of Pathologists of Australasia
www.rcpa.edu.au

CEO Dr Debra Graves
E-mail: debrag@rcpa.edu.au

While the views expressed are those of the authors, modified by expert reviewers, they are not necessarily held by the college.

Common Sense Pathology editor: Dr Matthew Meerkin
E-mail: mmeerkin@ozemail.com.au

Chief sub-editor: Jacqueline George
E-mail: jacqueline.george@reedbusiness.com.au

Australian Doctor
Editor: Nadine Meehan
E-mail: nadine.meehan@reedbusiness.com.au

Medical editor: Dr Lynn Buglar
E-mail: lynn.buglar@reedbusiness.com.au

Commercial director: Suzanne Coutinho
E-mail: suzanne.coutinho@reedbusiness.com.au

Graphic designer: Edison Bartolome
E-mail: edison.bartolome@reedbusiness.com.au

Production manager: Marlene Dickinson
E-mail: marlene.dickinson@reedbusiness.com.au

Cover: Computer artwork of anaemia. David Mack/Science Photo Library.

For an electronic version of this and previous articles, you can visit www.australiandoctor.com.au Click on Clinical and Library, then *Common Sense Pathology*. You can also visit the Royal College of Pathologists of Australasia's web site at www.rcpa.edu.au Click on Publications and Forms, then *Common Sense Pathology*.



Table 1. Haematological and biochemical changes in disturbances of iron status

	Early iron deficiency	Iron-deficient anaemia	Contraceptive pill	Anaemia of chronic disease	Thalassaemia minor	Iron overload
Haematology						
Haemoglobin	Normal (N)	↓	N	↓	N or ↓	N
MCV	N or ↓	↓	N	N or ↓	↓↓	N
MCH	N or ↓	↓	N	N or ↓	↓↓	N
RCC	N	↓ or N	N	↓	N or ↑	N
Marrow Iron	↓	↓	N	N or ↑	N or ↑	N or ↑
Biochemistry						
Iron	↓	↓	N	↓	N or ↑	↑
Transferrin	↑	↑	↑	↓ or N	N	N
Transferrin saturation	↓	↓	N	↓	N or ↑	↑
Ferritin	↓	↓	N	N or ↑	N or ↑	↑
Soluble transferrin receptor	↑	↑	N	N	N	↓
Zinc protoporphyrin	↑	↑	N	N or ↑	↑	N

Common indications for requesting iron studies

There are five common reasons to request iron studies because of suspected iron deficiency.

1. Investigation of fatigue.
2. Investigation of anaemia.
3. Investigation of someone with blood loss.
4. Nutritional assessment, particularly in early childhood and pregnancy.
5. Monitoring iron therapy.

Fatigue is a common reason for attending the doctor. Full blood examination is an important investigation in any patient with unexplained fatigue, as are iron studies in any women of reproductive age because of the high prevalence of iron deficiency in this group.

Groups of patients at increased risk of iron deficiency

Infants, growing children and pregnant women are at high risk of iron deficiency. Iron deficiency can develop by 2-3 months of age in premature infants but is more common in those older than six months who are exclusively breast fed, and in infants 9-18 months who are fed with cow's milk or low-iron-content formulas. Patients on haemodialysis are also at high risk due to dialysis-related blood loss and the demands on erythropoiesis from erythropoietin therapy.

Case study 1

A 21-year-old woman presented to her doctor with fatigue as her major complaint. She was of Mediterranean ancestry and had an unremarkable medical history and a normal diet. The results of a full blood examination, iron studies and subsequent haemoglobin analyses were:

- Haemoglobin 120g/L (reference interval [RI] 115-165g/L)
- Packed cell volume (PCV) 37.3% (RI 37.0-47.0%)
- Red cell count (RCC) $5.29 \times 10^{12}/L$ (RI $3.80-5.80 \times 10^{12}/L$)
- Mean cell volume (MCV) 71fL* (RI 80-96fL)
- Mean corpuscular haemoglobin (MCH) 22.7pg* (RI 27.0-32.0pg)
- Iron 11µmol/L (RI 7-27µmol/L)
- Transferrin 3.8g/L* (RI 2.0-3.6g/L)
- Transferrin saturation 14% (RI 13-47%)
- Ferritin 13µg/L* (RI 15-165µg/L)
- Haemoglobin A 94.6%* (RI >95.6%)
- Haemoglobin A2 4.7%* (RI <3.4%)
- Haemoglobin F 0.7% (RI <1%)
- Film: Anisocytosis, target cells, rod cells, hypochromia and microcytosis

*Outside reference interval





What is your interpretation of these results?

This patient has iron deficiency (low ferritin and raised transferrin) and beta-thalassaemia minor (raised haemoglobin A2). Low serum ferritin is the single most reliable marker of reduced iron stores in all clinical settings (children, adults and pregnant women). Serum iron is usually reduced in people with iron deficiency, although it was normal in this patient. The main problem with using serum iron measurements to assess iron deficiency is that levels are often decreased in people with normal iron stores.

Iron deficiency is often also associated with increased serum transferrin concentration. However, assessment using serum transferrin is limited because it is increased by high oestrogen levels in pregnancy and in women taking hormone replacement therapy or the oral contraceptive pill, and decreased in people with infection, inflammation, renal failure and malignancy.

Why is it important to establish the cause of microcytosis in a young woman?

Distinguishing the microcytosis and hypochromia of iron-deficiency anaemia from that of thalassaemia minor is essential because iron therapy is indicated in the former but contraindicated in the latter, unless there is concomitant iron deficiency (as in this case). The distinction is particularly important in pregnancy due to the potential impact on the fetus if both parents have a haemoglobinopathy. In people with thalassaemia, serum iron, transferrin saturation and ferritin all tend to be increased, so the low ferritin in this patient is particularly significant. The main distinguishing features of iron deficiency and thalassaemia are shown in table 1 (page 3).

What other investigations are required?

No other tests are needed in this patient although the cause of the iron deficiency should always be established. This is particularly important in groups where iron deficiency is less common, such as men and postmenopausal women, and causes such as blood loss from bowel cancer need to be excluded. Young women are often in a precarious

iron balance, so diet and menstrual history is important. Coeliac disease should be considered as one of the less common causes of iron deficiency, particularly if there is a family history.

Case study 2

A 42-year-old woman presented with pain in her hands and wrists. She had been taking increasing doses of NSAIDs to control the pain. Examination showed active synovitis affecting the wrists and metacarpophalangeal joints in both hands. Her blood results showed:

- Haemoglobin 87g/L* (RI 115-165g/L)
- PCV 31.0%* (RI 37.0-47.0%)
- RCC $4.28 \times 10^{12}/L$ (RI $3.80-5.80 \times 10^{12}/L$)
- MCV 72fL* (RI 80-96fL)
- MCH 20.3pg* (RI 27.0-32.0pg)
- Iron $4\mu\text{mol}/L$ * (RI $7-27\mu\text{mol}/L$)
- Transferrin 3.0g/L (RI 2.0-3.6g/L)
- Transferrin saturation 8%* (RI 13-47%)
- Ferritin $45\mu\text{g}/L$ (RI 15-165 $\mu\text{g}/L$)
- C-reactive protein $112\text{mg}/L$ * (RI $<10\text{mg}/L$)
- Rheumatoid factor $242\text{kIU}/L$ * (RI $<14\text{kIU}/L$)
- Film: Mild anisocytosis, hypochromia and microcytosis. See figure 1

What is your interpretation of these results?

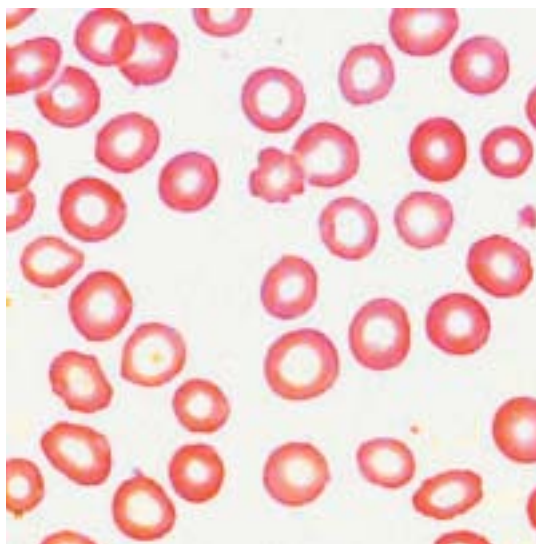
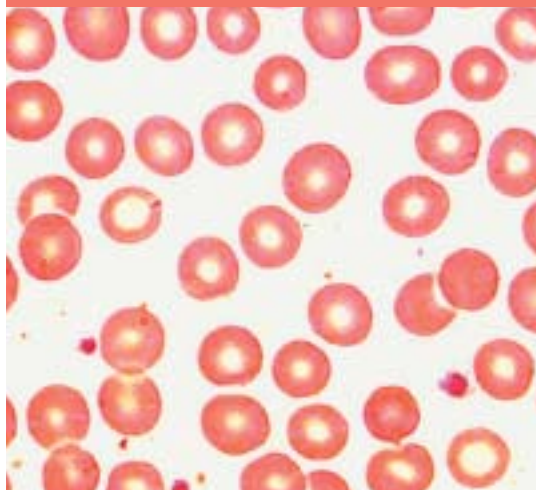
The changes suggest iron-deficiency anaemia in someone with recent-onset, active rheumatoid arthritis. The relatively low serum ferritin concentration is the most important finding. The main differential diagnosis in this patient is the so-called anaemia of chronic disease in which there are abundant iron stores but their utilisation is blocked by inflammatory cytokines. In a patient with anaemia of chronic disease uncomplicated by iron deficiency, the serum ferritin concentration is often greater than $100\mu\text{g}/L$.

How can you distinguish iron-deficiency anaemia from the anaemia of chronic disease?

Anaemia of chronic disease is the most common cause of anaemia in people aged over 50. It is usually normochromic and normocytic but may be



Figure 1. Normal blood film (top) and hypochromic, microcytic changes of moderate iron deficiency (bottom).



hypochromic and microcytic. Serum ferritin levels are typically decreased in iron deficiency and increased with inflammation, whereas serum transferrin levels are usually high in iron deficiency and low with inflammation (table 1, page 3).

It is important to remember that in people with iron deficiency and concurrent inflammation, serum ferritin levels may be toward the lower end of the reference interval but not frankly abnormal, because inflammation tends to increase ferritin levels. People with ferritin levels less than 50µg/L

may be iron deficient, although those with levels greater than 100µg/L are usually iron replete.

If the iron studies are inconclusive, options include:

- Repeating the blood tests in 1-2 weeks.
- Investigating for gut blood loss, regardless.
- Reviewing the patient after a 2-3 week trial of oral iron therapy.

If standard blood tests are unhelpful in haemodialysis patients the response to a dose of intravenous iron may be the most effective way to establish a diagnosis of iron deficiency.

What new tests are available for assessing iron status?

Soluble transferrin receptor Transferrin receptors are responsible for the uptake of transferrin-bound iron by cells in the bone marrow. They increase when there is increased red cell turnover (haemolytic anaemias and thalassaemia) and with iron deficiency, as the bone marrow seeks more iron. They decrease with iron overload. The concentration of soluble transferrin receptors in the serum reflects the number of these receptors in the bone marrow and can be used as a way of diagnosing iron deficiency. This test is not widely available but its theoretical advantage is that it is a good marker of iron deficiency that, unlike ferritin, is not affected by inflammation.

Red cell zinc protoporphyrin If there is insufficient iron to insert into protoporphyrin to make haem the body incorporates zinc instead, making zinc protoporphyrin (ZPP). ZPP has a distinctive fluorescent fingerprint that can be measured as an indicator of iron deficiency. Levels also increase with lead poisoning, disorders of haemoglobin synthesis and chronic inflammation.

Reticulocyte haemoglobin concentration (RET-He) and percent hypochromic red cells These measurements are available on some new automated haematology analysers and may be useful in diagnosing iron deficiency in the presence of inflammation. They are not widely available and

Australian
Doctor.

RCPA



Australian Government
Department of Health and Ageing



their true value will not be known until research has been completed.

Case study 3

A 61-year-old woman presented with fatigue. Abnormal liver function tests had been noted one year earlier but not investigated. Her latest blood tests were:

- Iron 42 μ mol/L* (RI 7-27 μ mol/L)
- Transferrin 1.9g/L* (RI 2.0-3.6g/L)
- Transferrin saturation 95%* (RI 13-47%)
- Ferritin 3241 μ g/L* (RI 15-165 μ g/L)
- Haemoglobin 155g/L (RI 115-165g/L)
- MCV 103fL* (RI 80-96fL)
- Bilirubin 14 μ mol/L (RI <20 μ mol/L)
- ALT 76U/L* (RI <35U/L)
- AST 59U/L* (RI <35U/L)
- ALP 133U/L* (RI 30-115U/L)
- GGT 119U/L* (RI <35U/L)

When should you look for iron overload?

Iron overload is often discovered as a chance finding when someone has iron studies looking for iron deficiency — as in this case. Blood tests may also be specifically requested looking for iron overload in the following settings.

- Investigation of someone with abnormal liver function tests, heart failure, diabetes or skin pigmentation.
- Family screening for hereditary haemochromatosis.
- Patients who have received multiple blood transfusions.
- Treatment monitoring in someone with iron overload.

What is the most likely cause of these abnormalities?

Hereditary haemochromatosis would be the best single explanation of these abnormal iron studies and liver function tests although a careful history is needed to exclude other causes. When considering the differential diagnosis, treatment with iron could cause an increased serum iron but would not cause such a spectacular increase in serum ferritin. Viral infection and medications could cause the abnormal liver function tests, as could alcohol

which can also cause macrocytosis.

What other investigations should be performed?

The single most important test in someone with results like this is testing the HFE gene for the common mutations seen in haemochromatosis, especially the C282Y mutation. Other tests may be needed depending upon the history. This woman was homozygous for the C282Y mutation, which is responsible for most haemochromatosis in our community. Other manifestations of haemochromatosis such as diabetes and arthropathy should be considered.

There are a number of important practical points about the diagnosis of haemochromatosis and the use of the HFE gene test. Raised serum iron and transferrin saturation are the most useful tests for the diagnosis of iron overload. Transferrin saturation cut-offs of either 45%, 50% or 55% have been used in different studies to detect haemochromatosis, the former giving better sensitivity and the latter fewer false-positive results.¹ The problem with transferrin saturation as a screening test is that it can be falsely increased by iron therapy and even, on some occasions, after a person has eaten an iron-rich meal. Transferrin saturation less than 45% is uncommon in people with haemochromatosis.

Serum ferritin is not useful in screening for haemochromatosis because it is normal for many years early in the disease. Often, the textbook features of haemochromatosis do not appear until the serum ferritin concentration exceeds 1000 μ g/L. Serum ferritin levels decrease with regular venesection. The patient in the case study subsequently had venesection at 1-2 weekly intervals over one year donating a total of 40 units of blood. Her serum iron, transferrin saturation, ferritin and liver function tests all normalised over this time.

The gene test for haemochromatosis, although simplifying family screening and reducing the need for liver biopsy in some people, has important limitations.

- Laboratories look for specific mutations of the HFE gene, usually C282Y, H63D and S65C. These mutations are linked with haemochromato-



sis in people with northern European ancestors but there are many people with haemochromatosis who do not have these particular mutations. They would be missed if gene testing alone was used to screen for disease.

- Some people with the C282Y mutation never develop haemochromatosis because other genetic and environmental changes are required to develop disease. Because it is assumed that people with high levels of serum ferritin and transferrin saturation will develop clinical disease eventually, treatment decisions are based on measurements of these parameters: it is not necessary to wait until signs and symptoms develop.
- As with all genetic tests, counselling must be provided before any testing is undertaken.

If a patient has an increased transferrin saturation the first thing to do is check whether they are taking iron supplements and to find out if

they have a family history of haemochromatosis. The iron studies should be repeated and the gene test requested if the transferrin saturation is still increased after cessation of supplements.

References

1. Schmitt B, et al. Screening primary care patients for hereditary haemochromatosis with transferrin saturation and serum ferritin level: systematic review for the American College of Physicians. *Annals of Internal Medicine* 2005; 143:522-36.

Further reading

- Andrews NC. Disorders of iron metabolism. *New England Journal of Medicine* 1999; 341:1986-95.
- Cook JD. Diagnosis and management of iron deficiency anaemia. *Best Practice & Research. Clinical Haematology* 2005; 18:319-32.

Conclusions

- Low serum ferritin, not low serum iron, is the most reliable marker of uncomplicated iron deficiency. If serum ferritin exceeds 100µg/L iron deficiency is unlikely (Table 2).
- Babies, growing children and pregnant women are at high risk of iron deficiency.
- Iron deficiency is a sign, not a diagnosis. You must establish the cause, particularly in men and postmenopausal women in whom iron deficiency is usually due to chronic blood loss.
- Raised transferrin saturation, not ferritin or haemoglobin, is the most useful marker for the early diagnosis of haemochromatosis and other iron overload syndromes (Table 3, page 8).
- Take care with the HFE gene test for haemochromatosis. Some people with the C282Y mutation do not have the disease and some people with disease do not have the C282Y mutation.

Table 2. Common misconceptions about iron deficiency

Misconception	Comment
Low serum iron equals iron deficiency	Serum iron concentrations commonly decrease during illness regardless of iron status. Iron levels may be lower in the afternoon than the morning. Ferritin is the best marker of iron deficiency.
Normal serum ferritin rules out iron deficiency	Some people with iron deficiency may have serum ferritin concentrations in the lower part of the reference interval. Ferritin is increased during inflammation and with liver disease.

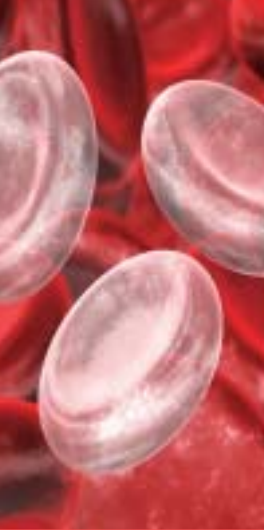


Table 3. Common misconceptions about iron overload

Misconception	Comment
Serum ferritin is a good screening test for haemochromatosis	Increased ferritin is a sign of established iron overload and is not seen in early disease. It is not safe to use this as a screening test. Increased serum iron and transferrin saturation are the tests of choice.
Polycythaemia is a feature of haemochromatosis	Full blood count is typically normal in haemochromatosis although there may be mild macrocytosis. Haematological examination does not have a significant role in diagnosis of hereditary haemochromatosis.
Increased serum ferritin equals haemochromatosis	Serum ferritin is commonly increased during inflammation and liver disease. If the transferrin saturation is normal a raised ferritin is likely to be caused by something other than haemochromatosis.
Homozygous C282Y mutations equal hereditary haemochromatosis	Many people with the classical haemochromatosis mutations do not develop disease. Conversely, some people with haemochromatosis do not have the C282Y mutation, presumably having some other mutation instead. Iron studies are essential for the diagnosis of haemochromatosis.

Actors in the drama of iron metabolism

Actor	Role
Iron	Highly sought-after star performer. Major role in haemoglobin where it has an ambivalent relationship with oxygen. Minor roles in other proteins, such as myoglobin and cytochromes. Destructive if allowed to wander freely about stage.
Transferrin	The chaperone that meets iron on its arrival in the bloodstream and carries it to its destination in the marrow. Keeps iron bound and out of trouble. Each transferrin can bind two iron atoms, although it carries 13-47% of its maximum iron carrying capacity on average. This percentage is increased in people taking iron and in those with haemochromatosis. Transferrin also becomes overloaded if the bone marrow is not working.
Haemoglobin	An important character with a four-part personality. Dependent upon the support of iron, folate and vitamin B ₁₂ . Normal career span is four months, although it may end early being lost in action, eg, in faeces. Sometimes injured by antibody attack or by being flailed by an artificial heart valve. Occasionally troubled by inherited character defects and problems with small size and reduced performance.
Ferritin	Very large, behind the scenes player with a major role in iron storage. On-stage visibility reflects the size of the iron stores, although levels may be increased by inflammation and liver disease.
Transferrin receptor	The companion of transferrin. Waits faithfully in the marrow for a brief meeting where they exchange iron. If the marrow is starved of iron, soluble fragments of the transferrin receptor are released in the blood in increasing numbers. Yet to gain an established role as a diagnostic marker of iron deficiency.

