Interpretation of iron studies

Introduction

Iron plays a vital role in the human body in oxygen transport, mitochondrial oxidative energy production, inactivation of drugs and toxins, and DNA synthesis (Munoz et al, 2011). Iron deficiency is one of the most common nutritional problems worldwide (Cook et al, 1994) and an understanding of how to determine a patient's iron status is consequently essential. Normally, a few inexpensive, rapid tests including the serum ferritin, serum transferrin saturation and the full blood count enable assessment of iron status. However, some of the terminology regarding this can be confusing, and understanding the results can be something both medical students and doctors struggle with.

Diagnostic methods for investigating iron status

Full blood count and blood film

The majority of iron in the human body is present in haemoglobin (Munoz et al, 2011). The full blood count is therefore vital when assessing iron status. The absolute value of haemoglobin, the mean corpuscular haemoglobin and the mean corpuscular volume should all be considered. A blood film should also be reviewed as there are typical red blood cell changes found in iron deficiency (Bain, 2006).

Assessment of storage iron

Ferritin is the primary iron storage protein, with the majority being found in the reticuloendothelial cells of the liver, spleen and bone marrow and the liver parenchyma (Munoz et al, 2011). In healthy subjects, the serum ferritin level correlates with iron stores (Jacobs et al, 1972). Normal concentrations range from 15–300 µg/litre, with women having lower levels than men. The bone marrow can also be stained for iron, giving an indication of reticuloendothelial iron stores as well as erythroblast iron (Agarwal and Prchal, 2009).

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Iron supply to the tissues

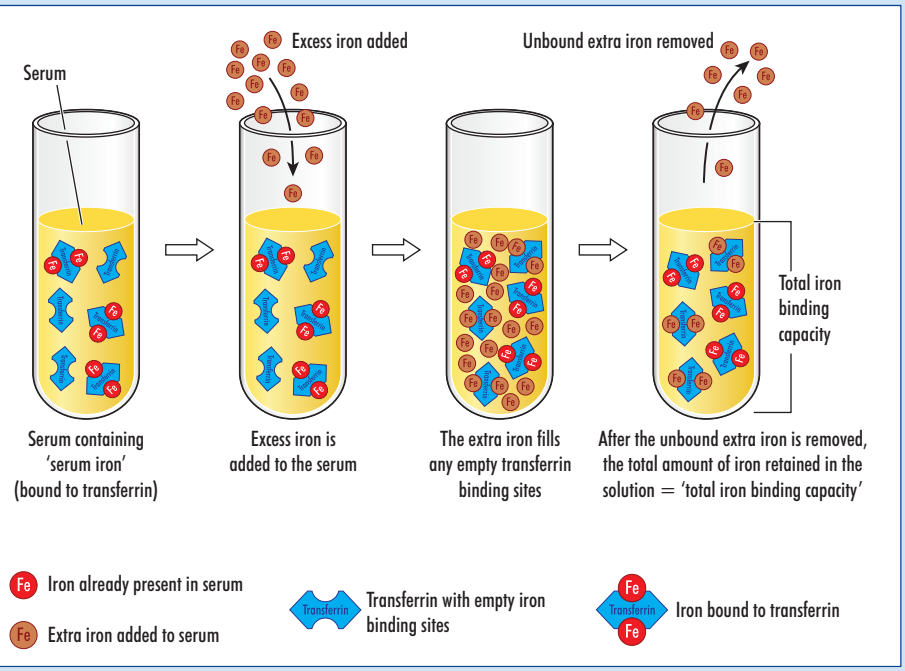
Iron is absorbed from the duodenum and then transported across the enterocyte by transport proteins before being taken up by transferrin, a single-chain polypeptide which transports iron within plasma (Munoz et al, 2011). Two values are typically measured in order to determine the iron supply to the tissues: the serum iron and the total iron binding capacity. 'Serum iron' is, in fact, a measure of the amount of iron bound to transferrin in the plasma (a typical normal value is 9–32 µmol/litre).

The total iron binding capacity is the total amount of iron capable of being bound in the plasma and is therefore an indirect measure of the transferrin concentration (normal range is approximately 45–66 µmol/litre). It is calculated by adding excess iron to serum in the laboratory, then measuring the iron retained in the solution, hence the name – total iron binding capacity (Worwood and May, 2012) (*Figure 1*).

Some laboratories also supply the unsaturated iron binding capacity, which can be calculated by subtracting the serum iron from the total iron binding capacity or measured biochemically. It is possible to work out the transferrin saturation by dividing serum iron by the total iron binding capacity and multiplying by 100; this figure is often routinely calculated by laboratories. Typical normal values for transferrin saturation are 20–50%.

Instead of measuring the total iron binding capacity, some laboratories measure the serum transferrin directly using an immunological assay (*Figure 2*). The normal range is 2.0–3.0 g/litre. There is generally a good correlation between the transferrin concentration and total iron binding capacity. Transferrin concentrations (g/litre) may be converted to total iron binding capacity (µmol/litre) by multiplying by 25 (Worwood and May, 2012).

Figure 1. Measurement of total iron binding capacity.



Changes in laboratory tests with different iron states

Iron deficiency

Iron deficiency is usually accompanied by a microcytic anaemia, defined by a haemoglobin level of less than 120 g/litre in women, and less than 130 g/litre in men with a mean corpuscular volume less than 80 fl. It is important to note, however, that iron deficiency can occur with a normal haemoglobin level and/or a normal mean corpuscular volume. In these situations, a low mean corpuscular haemoglobin or an increased red cell distribution width can suggest a mild iron deficiency. A low mean corpuscular volume in combination with anaemia is strongly suggestive of iron deficiency in the absence of a haemoglobinopathy, although anaemia of chronic disease can also cause a mild microcytosis. It is also important to remember that the mean corpuscular volume may be normal or even raised in iron deficiency when there is concomitant vitamin B_{12} or folate deficiency, excessive alcohol intake or myelodysplasia.

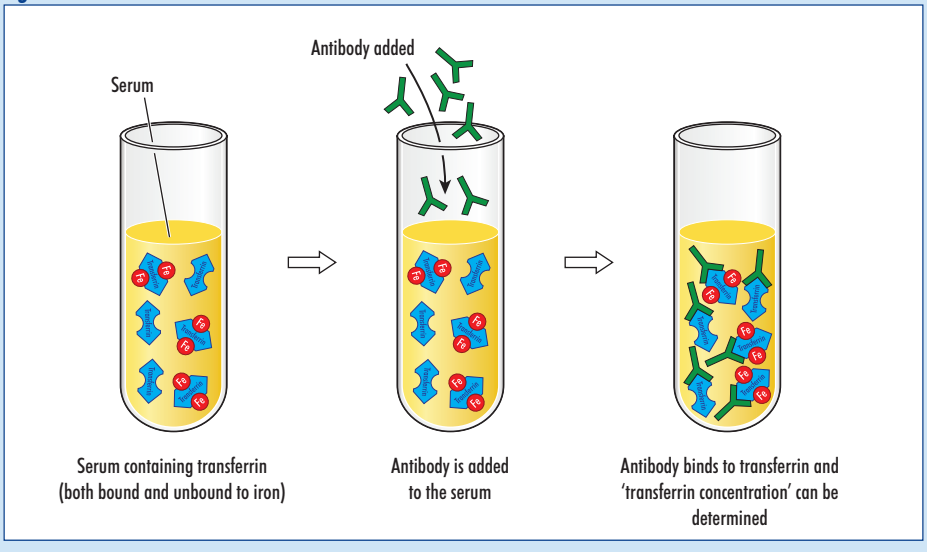
Inspection of the blood film in iron deficiency reveals hypochromic microcytic red blood cells, variation in red cell size and shape (*Figure 3*) and, in more severe cases, target cells and pencil cells (Bain, 2006). In iron deficiency, the platelet count can be increased, even in the absence of bleeding.

The most specific marker of iron deficiency is a low serum ferritin level (less than 15 µg/litre), and a low serum ferritin level always indicates iron deficiency (Lipschitz et al, 1974). However, serum ferritin is an acute phase protein, and consequently may be raised when inflammation accompanies iron deficiency, sometimes to very high levels (Agarwal and Prchal, 2009). It may also be raised in liver disease (Lipschitz et al, 1974). Consequently, serum ferritin levels may be normal or raised despite significant iron deficiency.

Iron deficiency leads to an increase in the total iron binding capacity or transferrin level and a reduction in the serum transferrin saturation. Both are characteristic of iron deficiency and can prove useful when the serum ferritin level is felt to be normal or raised as a result of inflammation or liver disease. However, the total iron binding capacity and serum iron concentration are also lowered by inflammation, are subject to significant fluctuations throughout the day and may be affected by eating (Worwood and May, 2012). A single one-off value may therefore not reflect iron supply accurately and ideally tests should be performed in the morning on a fasting sample.

The gold standard for the diagnosis of iron deficiency remains bone marrow examination with iron staining. In iron deficiency anaemia, marrow iron stores are depleted in both macrophages and erythroid progenitors. An adequate sample is required which may, on occasion, prove challenging (Hughes et al, 2004). In practice, it is often preferable to give the patient a trial of iron (either oral or intravenous) and then observe whether this causes a haemoglobin rise, rather than performing a bone marrow biopsy purely to diagnose iron deficiency.

Figure 2. Measurement of transferrin concentration.



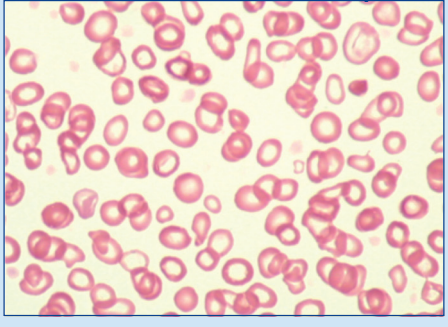
Anaemia of chronic disease

Anaemia of chronic disease reflects a state of functional, rather than true, iron deficiency: iron stores are normal but mobilization of these stores is insufficient to achieve normal erythropoiesis. An increase in hepcidin is the key mediator of this functional iron deficient state. Hepcidin is a small peptide (25 amino acids) predominantly produced in the liver. It regulates iron homeostasis by binding to iron transport proteins causing their internalization and degradation in lysozymes. It therefore acts to inhibit iron absorption, iron release from macrophages and iron transport across the placenta (Ganz and Nemeth, 2012). While measurement of the serum hepcidin level would theoretically prove useful diagnostically, a reliable test is not yet routinely available (Kroot et al, 2011; Thomas et al, 2013).

In anaemia of chronic disease, there is a mild to moderate reduction in the haemoglobin (normally no lower than 80 g/litre, even in severe cases) and the erythrocytes are usually normochromic and normocytic; sometimes in more severe anaemia they can become microcytic and hypochromic although the mean corpuscular volume is normally only slightly low, and almost never falls below 70 fl (Weiss and Goodnough, 2005). Inspection of the blood film may reveal rouleaux, but is otherwise unhelpful.

Biochemically, anaemia of chronic disease is characterized by a normal or raised ferritin level (the ferritin level may be very high, sometimes >1000 µg/litre), a low serum iron and a low total iron binding capacity or transferrin level. The transferrin saturation is typically low. There is usually evidence of inflammation with a raised C-reactive protein level and erythrocyte sedimentation rate (Weiss and Goodnough, 2005).

Figure 3. A typical blood film of iron deficiency demonstrating hypochromic microcytic red blood cells and variation in red cell size and shape.



Iron deficiency often accompanies anaemia of chronic disease (a typical example would be in inflammatory bowel disease) and can prove difficult to diagnose. A potentially useful biochemical test in such situations is the soluble transferrin reception concentration. This has been shown in some studies to be elevated in iron deficiency anaemia and iron deficiency anaemia with anaemia of chronic disease but not anaemia of chronic disease alone (Punnonen et al, 1997; Koulaouzidis et al, 2009). In practice, it is expensive, only performed in reference laboratories and is therefore rarely done.

More specialized tests available which are performed on red cells to assess the degree of iron-restricted erythropoiesis include the percentage of hypochromic red blood cells and the reticulocyte haemoglobin content (Thomas et al, 2013). Iron deficiency is implied by a percentage of hypochromic red blood cells >6% or a reticulocyte haemoglobin content <29 pg. These can prove useful in certain settings but are not routinely available and should be discussed with a haematologist before requesting.

Bone marrow examination in anaemia of chronic disease reveals increased iron in macrophages, with decreased iron in erythroid precursors, illustrating the state of a functional iron deficiency (Weiss and Goodnough, 2005).

Iron overload

Iron overload may be primary or secondary. Primary iron overload states (hereditary haemochromatosis or idiopathic iron overload disease) can result from mutations in several genes that then cause dysregulation of hepcidin synthesis (Roetto and Camaschella, 2005). The most common condition by far is hereditary haemochromatosis type I, which is caused by a mutation in the HFE gene, leading to deficient hepcidin production. Around 90% of patients with this condition are homozygous for the C282Y mutation (a cysteine to tyrosine substitution at amino acid 282 on the HFE gene); a second variant results from a histidine to aspartic acid substitution at amino acid 63 (H63D) which can form compound heterozygotes with C282Y. Secondary iron overload occurs as a result of chronic blood transfusion therapy required for a variety of conditions.

In iron overload, the full blood count parameters are normal (it does not cause polycythaemia), with a normal serum haemoglobin concentration and a normal mean corpuscular volume. The blood film is also unremarkable. The diagnosis is therefore based on biochemical parameters.

The fasting transferrin saturation is the most specific and sensitive test for primary iron overload states: patients with a value of >45% typically require further investigation (van Bokhoven et al, 2011). Ferritin values in excess of 200 µg/litre (women) and 300 µg/litre (men) strongly suggest iron overload in the absence of inflammation, excess alcohol intake and liver disease. When iron studies suggest primary iron overload in the correct clinical context, haemochromatosis genetic testing is indicated. In secondary iron overload, ferritin level is used to monitor the total iron burden and to guide treatment with iron chelation therapy: in thalassaemia major, for example, guidelines recommend maintaining the serum ferritin level below 1000 µg/litre (Cappellini et al, 2008).

In both primary and secondary iron overload, liver and cardiac magnetic resonance imaging is a useful non-invasive test to assess the degree of iron loading (Fischer and Harmatz, 2009). Liver biopsy may also be performed with chemical estimation of iron remaining the gold standard for determining iron overload (Bassett et al, 2011).

In thalassaemia intermedia which, by definition, does not require blood transfusions, ferritin level is less useful as a marker of iron overload. In this cohort of patients, abnormal iron accumulation occurs as a result of excessive absorption from the gut and for unclear reasons, the ferritin level may be only mildly raised (Taher et al, 2013) despite significant iron overload. It is therefore advised that such patients have an assessment of iron loading via imaging (liver magnetic resonance imaging) if their ferritin level is above the upper limit of normal (300 µg/litre; Taher et al, 2013). The changes in the iron studies associated with different iron states are summarized in *Table 1*.

Conclusions

Iron deficiency is a common problem, encountered daily in many hospital specialities and general practice. Its accurate diagnosis is important, both to allow appropriate treatment and guide further investigations to elucidate a cause. Tests vary from the most basic (a full blood count) to specialized biochemical assays

Table 1. Changes in iron studies in different iron states

	Iron deficiency	Anaemia of chronic disease	Iron overload
Mean corpuscular volume or mean corpuscular haemoglobin	↓	↓ (mild) or normal	Normal
Serum iron	↓	↓	↑
Total iron-binding capacity or transferrin	↑	↓ or normal	↓
Transferrin saturation	↓	↓	↑
Serum ferritin	↓	Normal or ↑	↑
Serum transferrin receptor	↑	Normal	↓
Bone marrow iron stores	↓	Normal or ↑	↑
Erythroblast iron	↓	↓	Normal

TOP TIPS

- Iron deficiency can occur with a normal haemoglobin level and/or a normal mean corpuscular volume.
- The only absolute statement that can be made with regard to iron studies is that a low serum ferritin level is always the result of iron deficiency.
- If there is a doubt about iron deficiency as a cause of anaemia, a trial of oral iron to see whether this increases the haemoglobin may be helpful as a simple diagnostic test.

and invasive procedures (a bone marrow). This article provides a framework for understanding these tests and how to use them in daily clinical practice. BJHM

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Conflict of interest: none.

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KEY POINTS

- Haemoglobin concentration, the full blood count and a blood film are important parts of iron status assessment.
- Ferritin is the prime iron storage protein. Measurement of the serum ferritin level accurately reflects iron stores in the absence of inflammation, excessive alcohol intake and liver disease.
- Transferrin is the main iron transport protein: transferrin saturation reflects iron supply to the tissues.
- A one-off value of serum iron and total iron binding capacity or transferrin (and therefore transferrin saturation) may not accurately reflect iron status as it fluctuates throughout the day and can be affected by inflammation and malignancy.
- Hepcidin levels are increased in anaemia of chronic disease and decreased in primary iron overload; however, measurement of hepcidin is not yet clinically useful.
- The most sensitive and specific screening biochemical test for haemochromatosis is the transferrin saturation: if haemochromatosis is suspected on the basis of biochemical tests, this can be confirmed by demonstration of the HFE gene mutation.