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Interpreting Serum Ferritin
Introduction

Iron homeostasis

Iron is an essential element for many living organisms. In humans, it is required for oxygen transportation (in haemoglobin and myoglobin) and electron transfer reactions.

Approximately two-thirds of the body's iron is found in erythrocytes, and a further 15% is in muscle and cellular enzymes. The remaining iron is excess to needs and stored primarily as ferritin or hemosiderin in the liver and within macrophages in the reticuloendothelial system. Iron recycling through the mobilisation of these stores means that most human diets can account for minor daily losses from the sloughing of epithelial cells or insignificant blood losses.

Absorption of iron from the small intestine and its release from macrophages is tightly controlled, as free iron has the potential to cause tissue damage through the production of reactive oxygen species. Maintaining low levels of free iron also aids resistance to infection, as bacteria constantly scavenge for iron from their environment for growth.

Hepcidin, a 25 amino acid peptide produced by the liver, is the principal iron-regulatory hormone providing the link between iron metabolism and innate immunity.1,2

Hepcidin production is stimulated by both iron loading and infection/inflammation — conditions where the body aims to limit the uptake of iron and its availability to invading organisms.

Hepcidin acts by binding ferroportin, a transmembrane protein involved in exporting iron from macrophages, erythrocytes and enterocytes. This interaction leads to ferroportin degradation.

This in turn leads to decreased dietary iron absorption, sequestration of iron into macrophages and decreased circulating iron concentrations. Stimulated production of hepcidin is also seen in the anaemia of chronic disease, where body iron stores are not deficient but simply not available for red cell production.

In hereditary iron overload syndromes, mutations in genes such as HFE cause a deficiency in hepcidin. It is likely that the liver, although exporting iron, has a greater ability to take up the increased plasma iron resulting in hepatic iron deposition.2

Ferritin and other iron studies

Serum iron studies typically include measurement of serum ferritin, iron, transferrin or Total Iron Binding Capacity (TIBC), and the calculation of transferrin saturation.

Iron studies are usually requested to diagnose iron deficiency or iron overload, but interpretation can be difficult because of the relationship shared by iron metabolism and inflammation.

Ferritin is an intra-cellular storage protein with the capacity to store up to 4000 iron atoms.

The concentration of ferritin in serum correlates well with the amount of storage iron as proven by phlebotomy trials. Hence, serum ferritin is a good marker of total body iron stores.

A low serum ferritin is almost only seen in iron deficiency.

In the presence of conditions such as inflammation, infection, malignancy (haematological and solid tumours), or liver or kidney disease, serum ferritin concentrations do not reflect iron stores alone and are typically higher than otherwise expected.

In addition, higher ferritin levels are seen with increasing BMI and post-menopause. In all these settings, a normal or elevated serum ferritin level does not exclude iron deficiency nor diagnose iron overload.

Serum iron concentration is a poor measure of iron status in the body.

In an individual, levels fluctuate significantly due to diurnal variation and fasting status.

Even when blood collection is standardised to morning samples in fasting patients, iron is an acute phase reactant and low levels may be seen as a consequence of acute inflammation.

Transferrin is often referred to as the circulating carrier protein for iron. In fact, this describes apotransferrin, which, when bound to either one or two atoms of iron, is then named transferrin.

Monoferric or diferric transferrin has high affinity for the transferrin receptor allowing cellular uptake by endocytosis. Iron is then utilised or stored by the cell and apotransferrin returned to the circulation.

Liver synthesis further contributes to apotransferrin levels so that high serum transferrin concentrations...
may be induced in iron deficiency or high oestrogen states (e.g., pregnancy, oral contraceptive pill use).

Low levels may be in response to iron loading or due to liver disease with poor synthetic function. Like serum iron, transferrin is also a negative acute phase reactant.

Transferrin saturation is a calculated ratio between serum iron and TIBC. Because of this, it is influenced by the analytical, physiological and pathological factors that affect these components.

TIBC may be measured directly or derived from measuring unsaturated iron binding capacity (UIBC) or transferrin. Measuring transferrin is generally more expensive for laboratories compared with TIBC or UIBC, but there is less variation in results between different assays.

Hepcidin, despite its importance in iron metabolism, is yet to have an established role in diagnostic testing and is not routinely available.

**Iron deficiency**

Iron deficiency is the most common nutritional deficiency worldwide, with anaemia only one part of the clinical spectrum.

It is now recognised that deficiency without overt anaemia is common, and can adversely affect growth, cognitive performance and behaviour in children and adolescents. It can also reduce immunity to infections, and decrease work capacity and performance in all age groups.

During pregnancy, iron deficiency with anaemia is associated with increased risk of maternal and infant mortality.3

In industrialised nations where poor nutrition and parasitic infestations are uncommon, the diagnosis of iron-deficiency anaemia in adult males and post-menopausal females warrants further investigation for a source of blood loss, particularly gastrointestinal malignancy.

### Case 1

Carolyn, aged four, presented to her GP for a pre-school entry health check. On questioning, her mother expressed concerns about lethargy and poor concentration. Blood tests revealed a normal full blood examination with no anaemia (Hb 124g/L) but the following serum iron studies:

- Iron 14μmol/L (5-25μmol/L)
- TRF 3.4g/L (2.0-3.5g/L)
- TRF.SAT. 16% (5-35%)
- Ferritin 6μg/L (20-200μg/L)

**Discussion**

The low ferritin (here in combination with a high-normal transferrin) is diagnostic of iron deficiency despite the normal serum iron, normal transferrin saturation and absence of anaemia.

Traditionally, serum ferritin concentrations less than or equal to 10-15μg/L have been used to diagnose iron deficiency based on high specificity for strict definitions of iron deficiency anaemia, but at the expense of poor sensitivity. Using a cut-off of less than or equal to 30μg/L has been shown to greatly improve sensitivity without much loss in specificity and using a cut-off of 50μg/L has been proposed to reduce the number of false negatives in the investigation of anaemic patients for colon cancer.4,5

Further support for a higher ferritin cut-off includes observations that levels of 30-50μg/L correlate well with iron deficiency by other measures such as bone marrow iron stores, hepcidin and soluble transferrin receptors, and increasing recognition that low level inflammation may be present in the general community.4,6,7

There is also now a greater appreciation for the spectrum of clinical iron deficiency and that supplementation, especially in children and adolescents, may reverse declines in outcomes such as cognitive functioning and physical performance.

When interpreting serum ferritin results, one should suspect iron deficiency in adults (including pregnant women) with values less than 30μg/L and in children with values less than 20μg/L.

The diagnosis of iron deficiency in the presence of inflammation is difficult. Even recognising inflammation is not straightforward because markers like C-reactive protein (CRP) are not always elevated, particularly in mild cases. In these situations, measurement of soluble transferrin receptors (sTfR) in serum may prove useful.

The expression of transferrin receptors on cell
membranes is up-regulated when body iron needs are increased such as in iron deficiency or in conditions of increased erythropoiesis such as polycythemia rubra vera. Serum concentrations of transferrin receptors are proportional to membrane-associated levels and are not an acute phase reactant.

High levels of sTfR are a sensitive indicator of iron deficiency but although testing is available, it is currently not Medicare-rebatable.  

The management recommendations for Carolyn were to commence iron supplementation and explore the possible causes for her iron deficiency.

Raised transferrin saturation may be the earliest indicator of hereditary haemochromatosis.

Oral preparations (3-6mg/kg elemental iron per day for children) are usually sufficient as first-line management of iron deficiency.  

Intravenous iron may be considered in selected patients, especially in the presence of iron deficiency anaemia, including those with demonstrated malabsorption or intolerance to oral iron, and where there is a clinical need for rapid iron supply.  

In infants and children, contributing factors to iron deficiency may include insufficient dietary intake, prematurity, increased requirements during periods of rapid growth, malabsorption (eg, coeliac disease) and blood loss.

Response to therapy should be assessed clinically and by re-measuring serum ferritin in six weeks.

Case 2

Theo is a 34-year-old teacher who presents to his GP with tiredness. His iron studies show the following abnormalities:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>40μmol/L</td>
<td>(5-30μmol/L)</td>
</tr>
<tr>
<td>TRF</td>
<td>2.2g/L</td>
<td>(2.0-3.2g/L)</td>
</tr>
<tr>
<td>TRESAT.</td>
<td>73%</td>
<td>(10-45%)</td>
</tr>
<tr>
<td>Ferritin</td>
<td>128μg/L</td>
<td>(30-500μg/L)</td>
</tr>
</tbody>
</table>

He was not taking any iron or multivitamin supplements.

Discussion

In the absence of excessive dietary iron or supplements, raised transferrin saturation may be the earliest indicator of hereditary haemochromatosis (HH), an autosomal recessive condition of progressive iron overload.

Men with HH may present with symptoms as early
as the fourth decade of life depending on the severity of disease. Women are generally diagnosed after menopause when menstruation and pregnancy can no longer offer protection against iron overload. Elevations in transferrin saturation typically precede a rise in serum ferritin, with levels greater than or equal to 45% being a sensitive marker of early disease.

Using this cut-off, 98% of patients subsequently found to be homozygous for the C282Y mutation of the HFE gene were identified without misidentifying any normal individuals in an Australian population.9

Early diagnosis and treatment are important in HH in order to avoid organ damage from iron overload and maintain normal life expectancy. It is one of the few conditions where genetic testing of asymptomatic individuals is suggested.

There is support within the MBS to offer genetic testing to patients with a first-degree relative diagnosed with HH, or individuals who demonstrate elevated serum transferrin saturation or ferritin on more than one occasion.

Theo was advised to repeat the serum iron studies on another occasion and this again showed a raised transferrin saturation. Subsequent genetic testing revealed that he was homozygous for the C282Y mutation of the HFE gene.

Currently, general genetic testing for HH only encompasses detection of mutations in the HFE gene, specifically C282Y and H63D.

In Australia, approximately 90% of people with symptoms of HH are homozygous for C282Y, but due to incomplete clinical penetrance, not all individuals with this test result will develop iron overload in their lifetime.

Long-term monitoring of serum iron studies and clinical review will distinguish between those who do and those who do not require treatment.

Of the other mutation combinations, only 1% of compound heterozygotes (individuals with both the C282Y and H63D mutations) will develop HH, but all individuals with this result should have their iron studies monitored every 2-5 years.

Individuals who are heterozygous for only the C282Y or H63D mutations, or homozygous for H63D mutation rarely develop clinical symptoms or signs beyond abnormal iron studies.10

It is important to note that some patients with the symptoms of haemochromatosis do not exhibit either of these genetic mutations and this may be due to the presence of other untested genetic mutations, environmental influences or a non-genetic cause of iron overload.

At present, Theo has normal liver function tests and no indicators of iron overload. If his serum ferritin increases over time, he will require regular venesection with the aim of keeping his serum ferritin below 50μg/L. Counselling should also be offered and consideration given to the genetic testing of family members.

Case 3

David is a 53-year-old man with a history of chronic alcohol abuse who presents to his GP with tiredness. His LFTs show longstanding abnormalities and his iron studies were also abnormal:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.protein</td>
<td>87g/L</td>
<td>(63-80g/L)</td>
</tr>
<tr>
<td>Albumin</td>
<td>41g/L</td>
<td>(34-45g/L)</td>
</tr>
<tr>
<td>T. Bilirubin</td>
<td>5μmol/L</td>
<td>(10-20μmol/L)</td>
</tr>
<tr>
<td>ALT</td>
<td>106 U/L</td>
<td>(5-40 U/L)</td>
</tr>
<tr>
<td>AST</td>
<td>33 U/L</td>
<td>(10-40 U/L)</td>
</tr>
<tr>
<td>GGT</td>
<td>387 U/L</td>
<td>(5-50 U/L)</td>
</tr>
<tr>
<td>ALP</td>
<td>231 U/L</td>
<td>(35-415 U/L)</td>
</tr>
<tr>
<td>Iron</td>
<td>11 μmol/L</td>
<td>(5-30 μmol/L)</td>
</tr>
<tr>
<td>TRF</td>
<td>2.2 g/L</td>
<td>(2.0-3.2 g/L)</td>
</tr>
<tr>
<td>TRESAT.</td>
<td>20 %</td>
<td>(10-45 %)</td>
</tr>
<tr>
<td>Ferritin</td>
<td>686 μg/L</td>
<td>(30-300 μg/L)</td>
</tr>
</tbody>
</table>

Discussion

A raised serum ferritin is not an uncommon finding in the general community and although most cases are not due to hereditary haemochromatosis, it is reasonable to consider genetic testing for HFE mutations in persistent cases.

Inflammation, infection, malignancy, hepatic and renal disease may all account for ferritin elevations. They
Elevated serum ferritin can also be seen with fatty liver, metabolic syndrome, obesity and diabetes mellitus.

Conclusion
A serum ferritin concentration less than 30μg/L in an adult or 20μg/L in a child indicates iron deficiency. The cause of the iron deficiency should be identified, and is particularly important in men and post-menopausal women, where this might be the first sign of bowel cancer.

It is important to recognise the limitations of this otherwise valuable test.

Ferritin concentrations may be increased by inflammation, liver and kidney disease, malignancy, obesity and age. These increases may obscure the decrease that one might expect in iron deficiency. Measurement of soluble transferrin receptors in serum may aid the diagnosis of iron deficiency in these settings.

In early iron overload, the only sign of hereditary haemochromatosis may be a transferrin saturation greater than 45%. Even though serum ferritin is a valuable marker of iron overload and a useful guide for therapy, it is often normal in early disease.

Acknowledgements
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References
Table 1: Algorithm for investigation of elevated serum ferritin

Elevated serum ferritin on multiple occasions

Geneic testing for hereditary haemochromatosis

C282Y homozygote
C282Y or H63D heterozygote
H63D homozygote
compound heterozygote (C282Y and H63D)

No HFE mutation identified

Ferritin >800-1000μg/L
Ferritin <800-1000μg/L

Monitor ferritin*
Refer to specialist (haematologist, gastroenterologist, physician).
Questions:
Cause?
Consider therapeutic venesection/ regular blood donation?
Consider liver biopsy?

Review history and examination for possible causes:
Exogenous iron (including blood transfusions)
Iron loading from ineffective erythropoiesis (thalassaemias, sickle cell anaemia, hereditary spherocytosis etc.) — Hb, blood film, Hb electrophoresis
Liver disease — LFTs and associated tests for cause.
Alcohol use >20g/day — repeat ferritin on reduced alcohol intake.
Chronic kidney disease — U+E, eGFR
Malignancy, infection, inflammation — CRP
Obesity, diabetes, metabolic syndrome — liver ultrasound (fatty liver), lipids

Hereditary haemochromatosis.
Commence therapeutic venesection. Monitor Hb (maintain >120g/L) and ferritin (aim <50μg/L)

Monitor ferritin*
Consider other causes for elevated ferritin

*Refer to specialist if ferritin steadily increasing or absolute level very high (eg. >1000μg/L)