**HEPCIDIN**

Compiled by Dr Shamin Mahabeer

**Introduction**

Iron is essential for the function of the oxygen-binding molecules haemoglobin and myoglobin, and many enzymes, including the mitochondrial cytochrome system. Cellular iron is mostly bound within iron protoporphyrin (haem) and iron-sulphur clusters (which serve as enzyme cofactors), or stored within the core of ferritin.

Without adequate iron, cells lose their capacity for optimum oxygen transport and energy metabolism. However, the redox activity of iron can also cause damage, primarily by the production of reactive oxygen radicals. Iron concentrations must therefore be closely regulated at both the cellular and systemic levels.

An important element in the maintenance of systemic iron homeostasis is effective communication between cells that absorb dietary iron (duodenal enterocytes), utilise iron (mainly erythroid precursors), and store iron (hepatocytes and tissue macrophages). The peptide hormone hepcidin interacts with the cellular iron exporter ferroportin, and is now recognised as the main regulator of systemic iron homeostasis and the principal hormone in iron regulation. Ferroportin is the major cellular iron exporter in the membrane of macrophages, hepatocytes and enterocytes.

**Synthesis and kinetics**

Hepcidin is predominantly produced by hepatocytes as a 25 amino acid peptide which is secreted into the circulation. Subsequent processing can result in the formation of 22 and 20 amino acids peptides, whose function remains largely unknown as they have almost no ferroportin regulatory activity. Other tissues that produce low levels of hepcidin include adipocytes, macrophages, renal tubule cells, and pancreatic β-cells. Circulating hepcidin is bound with comparatively high affinity to α2-macroglobulin and with low affinity to albumin. It is estimated that approximately 11% of hepcidin is freely circulating. Clearance occurs via the kidneys, and by means of cellular degradation with ferroportin at its sites of action.

Many different physiological and pathological conditions can influence hepcidin levels in the circulation (see Figure 1). Hepatocellular hepcidin synthesis will increase with inflammatory or infectious diseases, but decrease under conditions that require circulating iron levels to increase like hypoxia or anaemia.

![Figure 1. Clinical conditions known to influence circulating hepcidin levels (Reference 2)](image)
**Functions**

Hepcidin-25 is the major regulator of dietary iron absorption and cellular iron release. It exerts its regulatory function by counteracting the function of and inducing the internalisation and degradation of ferroportin, resulting in:

- Increased intracellular iron stores
- Decreased dietary iron absorption
- Decreased circulating iron concentration

In addition, hepcidin may also contribute to host defences. Hepcidin was originally identified as an antimicrobial peptide, and studies suggest that the bacteriocidal effects of hepcidin may occur locally in the phagosomes of infected macrophages. As iron is necessary for microbial growth, hepcidin may also contribute indirectly to host defences by reducing plasma iron concentrations.

**Hepcidin in iron disorders**

Measuring serum levels of hepcidin-25 can be valuable in identifying and differentiating between specific disease conditions. Abnormalities in hepcidin regulation have been implicated in at least two important clinical conditions: hereditary haemochromatosis and the anaemia of inflammation. Other potential applications are summarised in Table 1.

**Table 1. Most promising applications of serum hepcidin in diagnostic medicine** (Adapted from Reference 1)

<table>
<thead>
<tr>
<th>Disease condition</th>
<th>Expected hepcidin concentrations</th>
<th>Potential additional value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical hereditary haemochromatosis</td>
<td>Low (may be compensated by iron overload over time)</td>
<td>• Screen for the presence of haemochromatosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Prioritise genes to be investigated</td>
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<tr>
<td></td>
<td></td>
<td>• Predict which homozygous patients will be at risk for iron overload</td>
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<td></td>
<td></td>
<td>• Determine the phlebotomy interval</td>
</tr>
<tr>
<td>Iron-loading anaemias</td>
<td>Low (may be compensated by transfusional iron overload over time)</td>
<td>• Identify the most severely affected patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Predict and monitor (parenchymal) iron overload</td>
</tr>
<tr>
<td>Acquired forms of iron overload</td>
<td>Marginally increased (relatively low for the level of iron overload)</td>
<td>A marker of iron dysregulation</td>
</tr>
<tr>
<td>Iron-refractory iron deficiency anaemia</td>
<td>Inappropriately high</td>
<td>Screen for primary defect in hepcidin regulation</td>
</tr>
<tr>
<td>Inflammation and infection</td>
<td>High</td>
<td>Help to differentiate between anaemia of chronic disease and iron deficiency anaemia</td>
</tr>
<tr>
<td>Chronic kidney diseases</td>
<td>High (decreases upon erythropoietin treatment)</td>
<td>• Predict erythropoietin response</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Guide treatment with erythropoietin &amp; intravenous iron</td>
</tr>
<tr>
<td>Treatment with hepcidin agonists and antagonists</td>
<td>Depends on the disorder</td>
<td>Monitor and assess indications</td>
</tr>
</tbody>
</table>

a) **Hereditary Haemochromatosis (HH)**

Hepcidin levels are usually undetectable in conditions causing iron overload such as HH. The potential applications for hepcidin measurements where the diagnosis of HH is suspected include:

1. Screening for the presence of HH in patients with increased ferritin concentrations
2. Prioritisation of genes to be investigated (very low hepcidin concentrations point to juvenile HH forms)
3. Prediction of which C282Y-homozygous patients will be at risk for iron overload
4. Monitoring of phlebotomy treatment

b) **Hepcidin in infections and inflammatory diseases**

Hepcidin synthesis is induced by inflammation and infection. For example, malaria-associated anaemia is associated with low serum iron and increased hepcidin levels, causing iron withholding in the reticulo-endothelial system, and ultimately iron-restricted erythropoiesis. Conversely, in patients with Hepatitis C virus infection, there is increased transferrin saturation and serum ferritin levels, and low hepcidin concentrations.
c) Hepcidin in renal diseases

Even though hepcidin appears to be a promising supplementary diagnostic parameter for erythropoiesis stimulating agent (ESA) therapy, patients with renal insufficiency are a complex population, and several studies failed to demonstrate consistent results. The inconsistencies may be due to differing study parameters, including dialysis regimen, time of sampling in relation to iron and ESA therapy, and iron and erythropoietin dosage. Large, well designed studies regarding hepcidin concentrations in patients with chronic renal disease are currently in progress, and may help to resolve these discrepancies.

The assay

Lancet Laboratories now offers the Hepcidin-25 ELISA (enzyme-linked immunosorbent assay) based on the principle of competitive binding of hepcidin in the patient sample and biotinylated hepcidin to solid-phase-bound anti-hepcidin antibody. The assay can be performed on serum and plasma samples.

With all ELISAs there may be some cross-reactivity with the biologically inactive hepcidin-20 and -22 fragments, and thus overestimation of the true bioactive hepcidin-25 level. This is acceptable as long as the same assay is used to measure changes over time. At present, international task forces are working on harmonising assay outcomes, and to establish a traceability chain that will ultimately allow standardisation of hepcidin-25 measurements. These assay optimisations will assist to further define clinical decision limits.

In conclusion

Several studies on iron disorders published in the last decade highlighted hepcidin as a promising novel tool in diagnostic medicine as outlined in Table 1. The efforts of collaborative laboratory groups involved in the worldwide Hepcidin Harmonisation Study are contributing to the body of evidence that provides insight into the regulation of hepcidin, its functional properties, and the determination of ranges for clinical decision making.

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References
