Iron Age or New Age: Ironing out the Diagnosis of Anaemia of Inflammation from Iron Deficiency Anaemia

Mrs Nicola Svenson[1], Dr Russell Patmore[3], Miss Heidi Cox[2], Dr James Bailey[3], Dr Steve Holding[4]

[1.]Department of Haematology, [2.]Department of Clinical Biochemistry, [4.]Department of Clinical Immunology, Hull and East Yorkshire Hospitals NHS Trust, Hull, UK (e-mail: nicola.svenson@hey.nhs.uk)[3.]Queens Centre for Oncology and Haematology, Castle Hill Hospital, Cottingham, UK

Introduction

Iron deficiency anaemia (IDA) and anaemia of chronic inflammation (AI) are the most prevalent causes of iron related anaemia in subjects with gastrointestinal disorders contributing significantly to morbidity and mortality (Gomollón & Gisbert, 2009).

Diagnosis of IDA and AI is not always straight forward and currently a combination of several serum parameters (ferritin, transferrin, transferrin saturation, iron and C-reactive protein) is required (Thomas, et al., 2011). Subjects with a mixed aetiology can be difficult to interpret using traditional serum parameters, particularly in the presence of an inflammatory process.

Recently, hepcidin (a 25 amino-acid peptide hormone) has been identified as a regulator of haemostasis with levels being high in individuals with inflammation, and low in those with IDA (den Elzen, et al., 2013). Produced in the liver, hepcidin internalises ferroportin, blocking the release of iron from the Reticuloendothelial System and absorption of dietary iron, limiting iron availability for erythropoiesis (figure 1) (Weiss, 2009).

Changes in hepcidin concentration make it an ideal real-time marker of iron supply with haematologic response being seen within hours thus making it a useful marker (Thomas, et al., 2011). However, differentiating subjects with mixed aetiology is difficult with serum hepcidin values appearing within the normal reference interval (Theurl, et al., 2009).

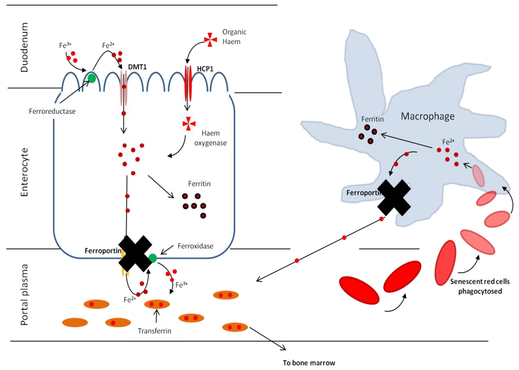

Figure 1. Metabolism of iron and effect of hepcidin on ferroportin and absorption of iron. Adapted from (Hoffbrand, et al., 2007 and Weiss and Goodnough, 2005).

Reticulocyte haemoglobin parameters can provide implied information regarding the adequacy of iron stores and demand for iron. Reticulocyte haemoglobin equivalent (RetHe) may help to distinguish subjects with IDA from AI with values <25pg suggestive of IDA and >25pg of AI (Canals, et al., 2005).

Thus, hepcidin in conjunction with RetHe has the potential to differentiate IDA from AI in cases of mixed aetiology replacing the traditional parameters (van Santen, et al., 2011).

Aims and objectives

- Evaluate the performance of a commercially available hepcidin-25 bioactive Enzyme Linked Immunosorbent Assay (ELISA).
- Investigate the possibility of differentiating AI from IDA/AI using the haematology parameter RetHe measurement in conjunction with the hepcidin value using the RetHe measurement to try to tease out which subjects have a likelihood of response to iron therapy.
- Appraise the potential of reducing the number of tests required during anaemia investigations using full blood count, serum hepcidin and RetHe measurements.

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Materials and Methods

- Seventy seven adult patients with gastrointestinal related disorders associated with anaemia in a secondary care setting using a traditional pathway of 6 tests (figure 2): Complete Blood Count (CBC), reticulocytes, serum ferritin, CRP, transferrin, and serum iron.
- Hepcidin concentration was measured using a commercially available ELISA method (DRG Diagnostic GmbH, Marburg, Germany), CBC and RetHe using a Sysmex XE-2100 CBC analyser (Sysmex Corporation, Kobe, Japan), iron parameters and CRP using Beckman Coulter platforms.
- Samples identified using the World Health Organisation (WHO) definition of anaemia and were collected during a six month period, with study assays performed on excess material after clinical analysis.
- Receiver Operator Curves (ROC) were used to determine diagnostic cut off concentrations (figure 3).

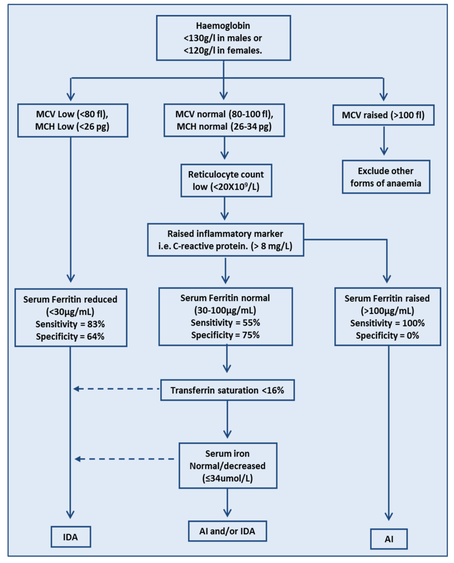

Figure 2. Current diagnostic testing pathway of 6 independent tests with serum ferritin used as the primary indicator of iron stores

Results

- Thirty six patients (77%) were shown to have IDA, 4 (5%) AI, 16 (21%) mixed aetiology and 21 (27%) normal iron status.
- Hepcidin correlated well with ferritin $R^2 = 0.79$, $p<0.0001$.
- The results were compared to traditional parameters with Receiver Operator Curves (ROC) used to determine diagnostic cut off concentrations (table 1).

Table 1. Sensitivity and specificity of serum ferritin and serum hepcidin used to determine diagnostic cut off values

Selected cut off values	IDA	AI
Serum ferritin 30.0µg/L	Sensitivity 83%	Sensitivity 55%
	Specificity 64%	Specificity 75%
Serum hepcidin 8ng/mL	Sensitivity 73%	Sensitivity 70%
	Specificity 72%	Specificity 67%
Serum hepcidin 40ng/mL	Sensitivity 98%	Sensitivity 25%
	Specificity 32%	Specificity 91%

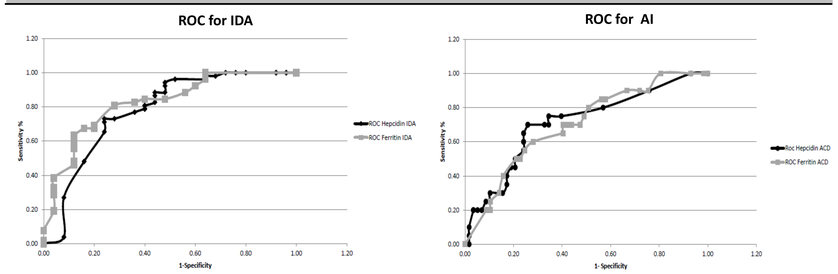

Figure 3. ROC curves for IDA and AI to determine sensitivity and specificity cut off values for serum hepcidin and serum ferritin.

- Sixteen subjects had mixed aetiology. Three had a RetHe <25pg indicative of true IDA and/or longstanding AI resulting in an iron deficient state. Thirteen had a RetHe >25pg suggesting AI with progression to IDA.

Discussion

The traditional pathway for the investigation of anaemia has long been established, currently consisting of 6 tests (figure 2) all of which are delayed markers (Thomas, et al., 2011). Ferritin was unable to distinguish IDA from AI in mixed aetiology situations. This gives rise to a new proposed 2 step pathway (figure 4) using 3 tests: CBC, RetHe and hepcidin differentiating IDA from AI in mixed aetiology cases indicating the probable cause of the anaemia. The RetHe value can then be used to predict the response to oral iron.

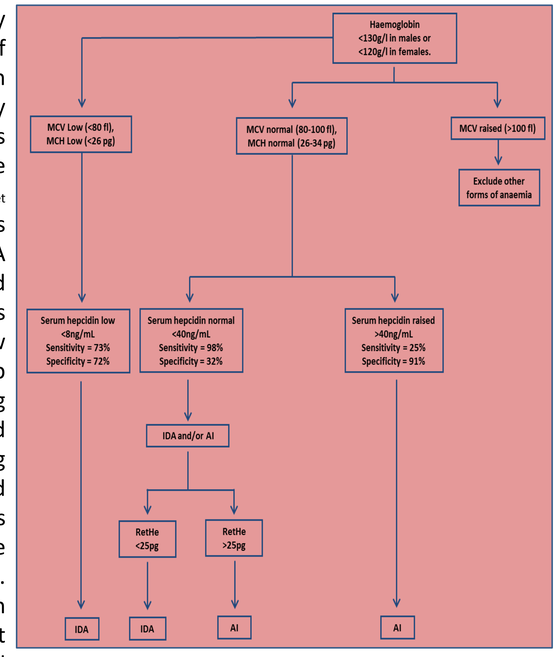

Figure 4. Suggestion of a new 2 step diagnostic pathway with serum hepcidin as the primary indicator and reticulocyte haemoglobin equivalent as the predictor of iron deficiency and response to oral iron.

Subjects with a serum hepcidin <40ng/mL with a RetHe <25pg are predicted to respond to oral iron therapy, whereas those with a RetHe >25pg may have reduced or no response. The advantage of serum hepcidin over serum ferritin is as a real time marker of iron status which can assist in early treatment interventions. Serum ferritin analysis still confers advantage over serum hepcidin due to rapid quantification by automated methods. The development of an automated ELISA method for serum hepcidin gives the potential for replacement of serum ferritin in the future reducing the number of traditional pathway tests.

Conclusion

Serum hepcidin may not yet replace serum ferritin as the preferred iron status marker, but in conjunction with RetHe it may distinguish mixed aetiology subjects. This offers the potential development of a clearer clinical pathway for investigation of difficult subjects, including reduction in the number of tests required during anaemia investigations and shorter diagnosis times. The advantage of hepcidin together with RetHe over traditional iron parameters is both as a real time marker of iron status and an indication of likelihood of response to iron therapy. The patient would benefit from a shorter recovery time, unnecessary testing, reduction in ineffective treatment and overall reduction in costs.

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"There are no relevant conflicts of interest to disclose"