REVIEW ARTICLE

Assessment of Iron Status - a Historical Perspective

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The importance of iron in the human metabolism has been recognised for millennia. During Roman times the usefulness of iron rich waters in dealing with symptoms of anaemia was well known and during the Middle Ages and later many folk remedies for ill-health, especially chlorosis, were based on iron - often in unpalatable forms. It was in the late 18th century in France that iron sulphate tablets were first prepared and these have been the mainstay of a simplistic approach to iron deficiency since that time. (see ***Fig. 1***)

The fundamentals of iron metabolism are simple. In a normal adult subject there are 3 g of iron bound up in circulating haemoglobin. At the end of the red cell lifespan iron is released at about 40 mg per day. This iron is transported bound to transferrin through the plasma to the developing red cells. Red cells require about 40 mg a day to make sufficient haemoglobin to make red cells red. The marrow produces 2-3 million new red cells per second. In some cases, this can increase tenfold. This erythropoietic cycle is the engine that drives iron metabolism.

Iron not involved in erythropoesis amounts to about 1 g, most of which is in the form of intracellular haemosiderin but a significant proportion will be in the huge array of haem proteins in the body. The link between all these processes is the transferrin iron pool. Only 4 mg, 1/1000 of the whole body iron, is in transit at any one time. An iron atom entering this pool will spend about 90 min there - a transient existence.

It was not really until the mid 20th century that an objective approach to assessing the state of iron metabolism in patients and their need for iron therapy was first established. The initial work of Witts[1)] and others took the size, shape and haemoglobin content of the erythrocyte as their starting point. It soon became evident however that not only was this highly subjective but was equally non-specific. It had been known for a long time that iron in the marrow macrophages could be stained using the Perl's Prussian Blue reaction. This was thought to represent iron that had been stored, presumably for later beneficial use as if the body was a careful saver putting something by for a rainy day. In truth the function of this stainable iron has never been established. Nevertheless, it soon became common practice to refer to this as "storage iron" and to this day this interpretation is still widely held. If it had been called "excess" or "quarantined" iron our understanding of iron metabolism might have been less confusing than many seem to find.

Attempting to use the stainable iron deposits in the marrow as an indicator of the need for iron therapy threw up a simple practical problem, when iron was present in abundance it could be easily visualised. The problem was when it was not there it was not easily seen! Any gradation between excess and absence was entirely subjective. In the early 1970's a study of the concordance between different eminent observers and of their intra-observer reproducibility showed such disarray that the participants refused to allow it to be published. It is disturbing to hear a younger generation refer to this art as a "gold standard".

In the early 1960's it became possible to measure iron reliably in serum[2)] and pathologists turned to the measurement of serum iron and its associated total iron binding capacity for an assessment of iron status. While it was clear that the serum iron concentration was often low in patients who would respond to iron therapy, and who were thus assumed to be iron deficient, this was a very variable parameter. Indeed, it varied significantly from day to day in all sorts of conditions. It was known that transferrin, the plasma transporter of iron, was not usually fully saturated. The degree to which it was saturated could be estimated by measuring the total iron binding capacity of the serum and expressing this as percentage transferrin saturation. The problem with all these measurements was the highly dynamic nature of the transferrin iron pool in plasma. This is in fact a minute proportion of the total body iron and in a highly labile state, turning over ten to twenty times each day. As a result any slight variation in the balance between inflow and outflow would produce an immediate increase or decrease in the residual level. This inherent biological variation made it very difficult to pin down an assessment of iron status from a single assay. The same remains true today.

The real clinical problem was - and remains - one of distinguishing between patients who would benefit from iron therapy and those who would not. The microcytic hypochromic anaemias associated with inflammatory conditions, infection and malignancy were not easily distinguished from those of frank iron deficiency. It was against this background that, somewhat serendipitously, Beamish[3)] first developed a workable method to detect ferritin in serum samples. Initially it had been assumed that this would be an iron containing protein and the early attempts to measure it were based on trying to detect iron associated with what turned out to be the ferritin protein. This was completely unsuccessful and led Addison[4)] and others to develop a radioimmunoassay for the protein. Under the leadership of the late Allan Jacobs they were able to demonstrate that its concentration in plasma appeared to reflect the level of mobilisable iron in the "stores" in the body as assessed by serial phlebotomy[5)]. This relationship was clearly indirect and could not be used to predict the level of mobilisable iron in an individual. Rather it showed that when the serum ferritin level was very low (< 12 µg/L) there was no iron that could be mobilised and that when it was normal (> 20 µg/L) there was. These two criteria are still true today and should be the basis on which all serum ferritin results are judged. (see ***Fig. 2***)

Fig. 1 *Poster advertising the use of iron preparations against anaemia, France, 1895. Reproduced with friendly permission of the Philadelphia Museum of Art.*

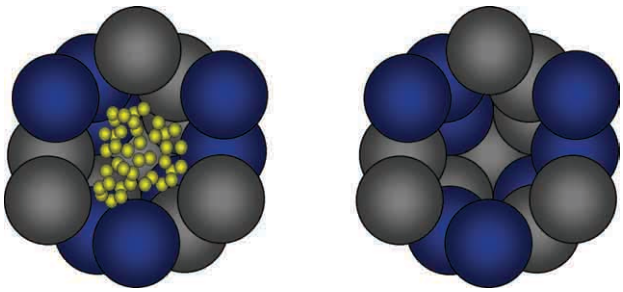

Fig. 2 *Rough schematic depiction of ferritin with (left) and without (right) iron stored in the central cavity.*

Ferritin consists of 24 subunits (L or H type) and can contain up to 4,500 iron atoms. Two types of ferritin are distinguished: Intracellular ferritin, which provides the iron storage, and serum ferritin, which usually contains very little iron.

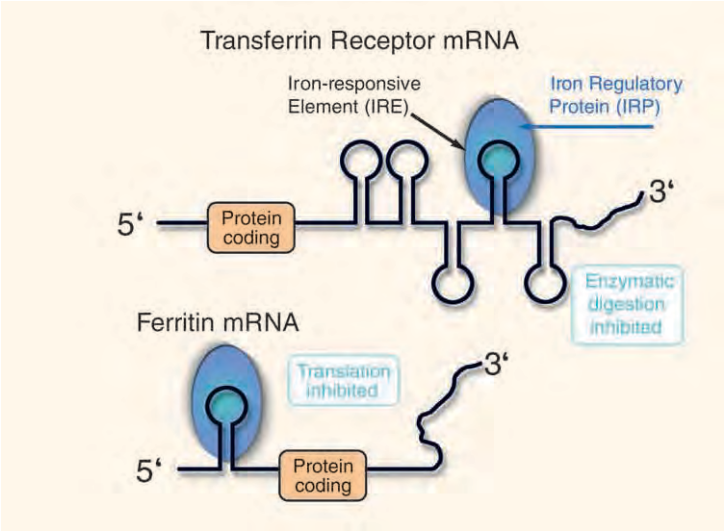

Fig. 3 *Regulation of transferrin receptor and ferritin expression by iron-responsive elements.*

Unfortunately no sooner had this rational state been reached that confusion began to creep in. The obvious clinical utility of a parameter that could pick out iron deficiency from the anaemias of chronic disease led to it being exploited commercially. However, it was not widely appreciated that there are two sorts of ferritin: intracellular and extracellular. Intracellular ferritin is largely found in cells of the reticuloendothelial system which provides a safe haven for iron that is not otherwise required. Extracellular ferritin (measured as serum ferritin) is a protein secreted from the surface of the reticuloendothelial cells but which contains virtually no iron. It does not appear to take any active part in iron metabolism and variation in its level seems to be a fortuitous association mediated through the intracellular labile iron pool of the reticuloendothelial cells. The concentration of iron in this vanishingly small pool appears to determine the rate at which serum ferritin is secreted into the plasma by those cells. In addition, considerable confusion has arisen because there are often occasions when intracellular ferritin is released into the plasma. Intracellular ferritin contains large amounts of iron and is not the source of the serum ferritin secreted by the reticuloendothelial - it normally has a purely intracellular existence. However, it may be released into the circulation, along with other intracellular components, in a variety of circumstances. Even a small release of intracellular ferritin will swamp the iron free serum ferritin secreted by the cell. Because the assay measures the total amount of ferritin protein, both serum and intracellular, in the plasma then the total ceases to be a reflection of the level of iron in the stores.

Considerable confusion has been added lately by the assertion that serum ferritin is unreliable because it acts as an acute phase reactor. This misapprehension has arisen because in the circumstances in which there is an acute phase reaction, as the result of infection, inflammation or malignancy, then the serum ferritin also rises. However, these observations are most readily explained as the result of the suppression of erythropoiesis which these conditions induce through the action of TNFα. Suppressed erythopoiesis will lead to a diminished requirement for iron by the developing red cells in the face of the release of normal amounts of iron from the catabolism of effete red cells. Iron which is no longer required for red cell production will be put into quarantine out of harm's way in the reticuloendothelial cells. This in turn will stimulate increased secretion of serum ferritin from those cells. The rise in serum ferritin concentration in these circumstances does not represent a direct acute phase reaction but is simply a consequence of the diversion of iron as a result of suppressed erythropoiesis. The rise in serum ferritin in these conditions remains a valid reflection of iron in the stores.

There seems to be a widespread view that there is a golden parameter which will give an assessment of iron status. This is mistaken. Iron metabolism is not a single entity and there is no single parameter which will assess its singular status. Iron levels in each of the iron pools in the body can vary, to a degree, independently of the other. Moreover, often the real clinical question is "does this patient need iron therapy, and if yes, in what form and why?"

A small amount of blood is lost from the gut every day in all people and there is a need to replenish that blood loss iron. The iron that is absorbed is not the major component of the iron required for erythropoiesis; it is just a top-up of the normal losses. The demand for this is largely controlled by the erythropoietic activity of the marrow itself. The amount of iron that can be absorbed however is limited by the solubility of the iron in the diet. Iron which is absorbed but which is not directly required for haemoglobin synthesis or other minor metabolic haem requirements will be largely deposited in the reticuloendothelial system. Here the iron, which if left

unattached and available in the body would be highly toxic, is held in a safe and unreactive form. This quarantined iron is first put out of harm's way in intracellular ferritin. As the central core of the ferritin molecule becomes full of iron, the molecules begin to coalesce and their protein shell breaks down. The net result is the formation of insoluble ferric hydroxide complexes in the reticuloendothelial cells. These are the compounds which give the dramatic blue with the Perl's Prussian Blue stain. This quarantined iron is largely unavailable for current metabolic activity. This means that it cannot rapidly be drawn upon if the need should arise.

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The three main iron pools in the body can be assessed individually but you cannot extrapolate from one to the other. The amount of iron in the red cell pool is most readily, but indirectly, assessed by the haemoglobin concentration. The iron in the transit pool at any one instant may be assessed by measuring serum iron (sometimes expressed as transferrin saturation) and the amount of iron quarantined in the reticuloendothelial "stores" may be assessed by measuring serum ferritin. It is unfortunate that a rational approach to the assessment of iron status seems to be in retreat, possibly because of the ready availability of serum iron and transferrin saturation measurements from the biochemistry laboratories. In truth this has added nothing but confusion to what was a relatively clear situation.

The introduction of recombinant human erythropoietin (EPO) to medicine in the late 1980's brought about a significant change in our understanding of the assessment of iron metabolism and iron therapy. In the earliest reports by Eschbach[6] it was clear that EPO could have almost miraculous effects on the production of red cells in patients with the anaemia of chronic renal failure. However there was a subset who failed to respond to this new therapy. They appeared to require iron therapy even though their serum ferritin, and hence iron stores, were normal or even significantly raised. This need for iron in the face of adequate iron "stores" was termed Functional Iron Deficiency[7]. Assessing the need for iron therapy in such patients was not straightforward but attempts were made to use the serum iron concentration and transferrin saturation as indicators. In the same meeting in which Van Wyck came to the conclusion that serum iron and transferrin saturation were not reliable, and that there must be a better way forward, Macdougall et al[8] reported the results of the first use of percentage red cell hypochromia. The ability to assess the percentage of red cells which had a suboptimal concentration of haemoglobin within them in a reliable way had just become practical using the H1 automated cell counter from Bayer (now Siemens). The realisation that this might allow us to distinguish the marrow's needs for iron from the level of iron stores had "dawned" one wet Tuesday afternoon in Birmingham and this was confirmed by a subsequent multicentre study[9]. From that point on the use of percentage hypochromia to indicate the need for intravenous iron therapy blossomed. This simple investigation and the administration of intravenous iron in appropriate patients transformed the use of EPO in patients with chronic renal failure, such that it became a standard part of the procedure. In truth it was a pretty crude measure and it was subsequently followed by more sophisticated parameters. In particular the assessment of the haemoglobin content of the reticulocytes themselves became a possibility. Within the Bayer (Siemens) family of machines this was known as CHr (reticulocyte haemoglobin) and for a time this was the only such measure available. Latterly however the SYSMEX family of instruments have developed to provide comparable quantitative measures of reticulocyte haemoglobin content, called Ret-H_e. CHr and Ret-H_e are numerically identical.

It is unfortunate that the lessons that were learned in renal medicine are only slowly transposing to other areas in which Erythropoietic Stimulating Agents (ESA's) may have beneficial effects. Nonetheless it is likely that once institutional inertia and prejudice have been overcome the power of ESA's to deal with anaemia will be recognised and that by using reticulocyte haemoglobin parameters to identify the need for iron support the full potential of this therapy will be realized.

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